

### Instructions Figure 1: Sequence Analysis and Alignment

Submit to Gradescope by 11pm 9/24/21

Figure 1 is a visual representation of how the EFO7 mutation affects the *spastin* gene region.

It should address the questions – What nucleotide changed? How is this predicted to affect the protein? Is the effect likely to be important for Spastin function and in what way(s)?

This is an opportunity to be creative and artistic! Brainstorm the most effective methods for portraying a gene product in a professional, visually appealing, and scientifically accurate way. Your audience is a scientifically literate undergraduate, so define any acronyms and explain what your result means as if to one of your peers.

Panel A should include the location of the nucleotide and/or amino acid change and any relevant protein domains –  
(e.g. If K at position 488 changed to R, write “K488R.”)  
Number the amino acids according to the spas-PA isoform.

Panel B should provide at least one potential reason this mutation is important to Spastin function or a mechanism that might be disrupted. The content of this figure is the most open-ended and allows you to follow questions that you find interesting: HSP patients, phylogenetic trees, biochemical structures, etc. For instance, is the altered amino acid within the *Drosophila* Spastin polypeptide sequence conserved through evolution? Does the altered amino acid disrupt an enzymatic domain, a protein-binding motif, a phosphorylation site (or some other regulatory target)?

Figure legend A single figure legend should incorporate the information in all figure panels. The first sentence should be a **strong take-home message**. Then each panel is discussed individually, including details of the image or cartoon (definitions of shapes, colors, symbols, scale bars, etc).

Ridiculous Example:

Figure 1: People experiencing chronic sleep deprivation demonstrate increased grumpiness. A) Images of facial expressions show an increase in scowling. Arrows indicate wrinkled brow, while arrowheads point to downturned mouths. Red number reports the change in the angle of eyebrow from neutral. B) Quantification of grumpiness measured in complaints per hour (cph). Error bars represent standard error. \* indicates statistical significance ( $p < 0.01$ ).

Literature Cited should include full references for any primary literature or prediction algorithms used. Websites for prediction algorithms generally provide references at the bottom of the page.

Submission- Do not include your name anywhere on the document or filename. We grade these anonymously in Gradescope. Include your unique id in the file name.

### **Instructions- Figure 2: Larval Neuromuscular Junction**

Submit to Gradescope by 11 pm 10/22/21

Figure 2 should incorporate images of the NMJs, as well as quantification of the neuronal branching phenotypes. Ideally, you will choose images that exemplify the conclusions made after quantification.

-Panels of HRP staining (one panel per genotype)

-Panel of the average terminal bouton number per hemisegment graph.

Images: False-color the red HRP image by copying the red channel into the blue channel to produce a magenta image. (Adobe Photoshop or GIMP software) Remove the green channel, because DLG obscures the tiny boutons. [If you get a completely green image when you do this, check that the background color is set to black (not white).]

Include a scale bar of 10  $\mu\text{m}$ .

Quantification: Use the shared class dataset to calculate the Average Terminal Bouton Number per hemisegment for each larva and for each genotype. Construct a graph with 3 "layers." Use a lighter/less prominent symbol to represent the raw terminal bouton number for each hemisegment. Use a more prominent symbol to represent the average per hemisegment for each larva (Not by student. Are the numbers similar among students or do we need to normalize them in some way?). Use a dark line or bar height (with error bars = standard error) to represent the average per hemisegment across all larvae of a particular genotype. Perform a statistical analysis (ANOVA) to determine significant differences from controls. Add a symbol to represent statistical significance.

Figure legend: Begin with a strong take-home message. Define genotypes, antibodies, scale bar, colors, symbols, error bars, statistical analyses, and p values.

Results: This section is a cohesive reporting of ALL the experiments to this point (include both Sequence Analysis and Neuromuscular Junction). [Note- you do NOT have to update Figure 1/ Figure 1 legend.] The Results section should

- 1) set up the rationale / goal of each experiment,
- 2) describe the results and what they mean,
- 3) refer to each Figure as you discuss each section,
- 4) make any conclusions about the severity of EFO7.

**Feedback:** Write a short note describing how you addressed the feedback from Figure 1.

**Submission:** Do not include your name on the document. Save the file as a single .pdf with your unique id in the filename. Upload to Gradescope.

General Rubric for this assignment:

Images			Graph					Figure Legend				Results			Feedback	Submission		
Clear images, cropped, aligned, false-colored	Scale Bar	Panel labels match up	X-axis labels include sufficient genotype information	X axis categories are in logical order (controls first, mutants in ascending numerical order)	Y axis label with units	Y axis range and markers are appropriate	Error bars and statistical significance are clearly indicated	Sample size (avg # boutons per larva) is clearly indicated	Strong take-home message as first sentence	Panels / Genotypes described individually	Colors, Antibodies, Scale bar, Symbols (bars, lines, asterisks, etc.) defined	Statistical test, error bars, p values for significance defined	Purpose of experiment and conclusion are clearly expressed.	Sets up rationale / goal for each experiment	Reports Results and references Figures as each is discussed	Makes a conclusion about severity of the alleles	Addresses comments about previous submissions	followed submission directions