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Data-Savvy Scientists: Utilizing Data Figure Format for Iterative Cell Biology Laboratory Assessments Involving Zebrafish

Jenean H. O'Brien

University of Wisconsin - Superior, Department of Natural Sciences, Belknap & Catlin, Superior WI 54880 USA
(**jobrie13@uwsuper.edu**)

Data figures are the currency of science communication. Visual representations of experimental results are used to 'sell the story' of our own research to fellow scientists and even to the general public. Further, we use these visual representations to analyze the experiments performed by others, to decide whether we 'buy it.' Therefore, learning how to appropriately design and interpret data figures is critical to the education of future scientists, and also to non-scientist consumers. Here, we present a series of laboratory activities from our upper division Cell Biology course in which students are assessed on their ability to create data figures from their own experimental results. These experiments utilize zebrafish to perform immunofluorescent (IF) staining and Western Blotting (WB). First students perform IF experiments to investigate their hypotheses and develop Figure #1 to share their imaged results. After receiving instructor feedback, students perform WB experiments to investigate the same hypotheses and develop Figure #2. The goal of this iterative data figure design is to expose students to the challenges of effectively presenting their data to communicate what they see and to create awareness of how science consumers may inaccurately interpret these displays, while providing students an opportunity to improve on their process. Additionally, these exercises demonstrate how multiple techniques can be utilized to explore similar questions. Examples of how these data figure assignments have been incorporated into other course aspects, including team-based problem sets and exam questions are included, along with a brief review of additional laboratory exercises that have utilized this assessment format in Genetics and Cell Culture courses.

Keywords: molecular biology, inquiry-based learning, immunofluorescent stain, Western blot

Introduction

Biology education research repeatedly demonstrates the most effective ways to train our future scientists involve having students *do* science. We are working to get students at all education levels out of their chairs and doing experiments. Whether participating in large-scale Course-based Undergraduate Research Experiences (CUREs) in BIO 101 or tracking which foods mold the fastest for an elementary school science fair, our students are collecting data. And they share it - visually. Social media apps are filled with snaps of fizzing baking soda volcanoes, selfies of students in waders for Ecology class, and cell phone photos of Gram stains taken through the microscope lens. It seems we could capitalize on this love of (over)sharing to teach our students that what comes after data collection in the science world is sharing the data. How many of your peers' lab notebooks have you actually read? The stodgy lab reports that meticulously document student work in an undergraduate lab course are not an authentic representation of how scientists share their data with each other. Further, in this age of expedient media and cell phone obsession, we all encounter data daily, in political polls (bar graphs), opinion surveys (pie charts), and even weather reports (data tables). All of us use these visual data representations to decide whether we 'buy it.' This is data communication, and it mimics how we attempt to 'sell the story' of our own research to fellow scientists. Beyond the words, we view and assess our colleagues work visually as well, with data figures, including images of our results. *Doing science* involves sharing visual results.

After attempting to visually represent our interpretations of experimental data in easy-to-understand figures, we scientists have realized how tricky this can be. The importance of taking representative images, of choosing the right scale for graph axes, of adding arrows to point to areas of interest on images, maps, and Western blots, becomes more obvious when you are designing your own data figures. We have all seen it done poorly, and experienced the gross misinterpretations or confusion that can follow. Therefore, learning how to appropriately design and interpret data figures is critical to the education of future scientists, and also to non-scientist data consumers in our general education courses. This was the impetus for incorporating data figure design into our laboratory classes. Saying to the students, "You have collected the data, analyzed it; now, how do you show us what it says, what it means?" allows students the opportunity to take the next step in science communication.

When thinking about how to get students sharing their science, consider that the exercise of designing data figures could be incorporated into almost any biology laboratory course where data is collected. The laboratory exercises included here are from our Cell Biology course, which utilizes zebrafish as a model organism. Our goal was to provide students with an opportunity to learn two common techniques in the Cell/Molecular Biology field, the immunofluorescent stain (IF) and the Western blot (WB), while performing inquiry-based experiments from which they would generate their own data to share in figure format. Additionally, we aimed to emphasize how two different techniques can be used to test the same hypothesis. This is what we refer to as the iterative experience. The students have one main hypothesis throughout the semester-long laboratory course, and utilize both IF and WB experiments to test it. Similarly, we encourage others to consider designing a laboratory experience that allows students to try multiple techniques to test the same hypothesis.

At first, this course design was daunting to organize and implement. Therefore, we provide you with our materials for the semester-long experience to aid in your prepping and planning. We anticipate these materials will be of interest to readers looking to perform IF and/or WB experiments in laboratory courses either with or without zebrafish; in addition to readers curious about how we instruct students on data figure design and assess the results. Hopefully, reviewing our experiences sparks ideas for how to help your students share their data too – no matter the course topic or level.

Laboratory Exercise Overview

This laboratory course meets once a week for two hours and is aimed at the junior and senior undergraduate level. Working in groups of three to four students allows conservation of reagents and peer training opportunities, while individual assessment is derived from data figure design. The introduction to the overall project, data figure design instructions, and notes for instructors are included below. The following can be found in the Appendices: laboratory instructions for students (Appendices A-B), materials needed (Appendix C) and assessment materials (Appendix D).

For three weeks (Table 1) the students work in groups to perform their IF experiments, then each student individually designs their own Data Figure #1 based on the results. The first data figures primarily contain IF images, occasionally a few advanced students have enough data to quantify and add graphs. The instructor provides feedback on this first figure before the WB experiment begins, to allow students time to process and incorporate recommendations as needed into their experimental design. For example, many students could benefit from aligning certain images from their IF experiments next to each other for comparison, and it helps to consider how that might affect the decision of which samples are loaded next to each other in the gel lanes for the WB.

The WB experiments are then performed across six weeks (Table 2) in the same groups, followed with each student individually creating their own Data Figure #2 containing their new results. The second data figures contain images of a Ponceau stained membrane and the chromogenically-stained WB results. In class, we discuss how densitometry could be used to quantify these results, however to date this method has not been included in students' data figures.

Also, a surprisingly simple piece of equipment has really garnered student excitement for these labs. Moticam X cameras attach to the eyepiece of most conventional microscopes and live stream to six WiFi-enabled devices. So while one student controls the microscope, several other students can simultaneously 'see' through the microscope and take their own images on cell phones, tablets or laptops. In addition, the live view can be projected onto a large screen for class-wide observations, if desired.

Additional Ideas for Utilizing Student Data Figures

We have modified student-designed data figures for use in additional course exercises. When students were tasked to review each other's data figures within a group and discuss similarities/differences, they almost instantly became

aware of how the same data collected by the group could be displayed differently by each group member. Further, students noticed that some of these differences affected interpretation – both positively and negatively.

To try to emphasize these conclusions, we have created worksheets that include de-identified data figures taken from previous semesters that are designed to lead students to make specific suggestions for how to improve the figures. The format for these worksheets provides a data figure, asks an interpretation question that is hindered by how the data is presented, has the student identify what is missing/presented inaccurately, and finally asks for explicit instruction for improvement.

Additionally, student-generated data figures have been used directly as a data source for team problem sets or for exam questions. The goal here is to emphasize to the students that they are capable of creating quality data figures, available for consumption by their peers.

The creation of data figures as an assessment technique for iterative laboratory experiences has been incorporated into other courses we teach as well. In a Cell Culture Laboratory course, we have repeatedly tested the same hypothesis across one semester utilizing cell counting, hematoxylin & eosin (H&E) staining, IF for proliferation and apoptosis markers, and WB analysis for the same proliferative and apoptotic targets. In Genetics laboratory, students setup zebrafish test-crosses, phenotype offspring and perform a Chi-square analysis to test an inheritance pattern hypothesis. Students then test the same hypothesis with molecular techniques through finclipping, DNA isolation and PCR analysis. We encourage you to consider the variety of techniques performed in your field that could be used iteratively in an undergraduate biology course to test one hypothesis, and to start having students design their own data figures. Sharing our results is part of *doing science*, so that's what we want students to learn and model.

Student Outline

Objectives

To experience working with a live model organism (zebrafish).
To perform an immunofluorescent stain (IF).
To perform a Western blot (WB).
To develop and test a hypothesis (utilizing zebrafish, IF and WB).
To organize your data into figure format (Figure #1 – IF, Figure #2 – WB).

Introduction

You have the following materials available. Think about what kinds of questions you could ask with these available materials using immunofluorescent (IF) and Western blot (WB) techniques. With guidance from your instructor, your group will develop a hypothesis that is testable with these materials.

Zebrafish embryos at three timepoints:

- 24 hours post fertilization (hpf)
- 48 hpf
- 72 hpf

Primary antibodies:

- phospho-HistoneH3 (rabbit)
- KI67 (mouse)
- cleaved caspase-3 (rabbit)
- tubulin (mouse)
- GAPDH (rabbit)
- Myosin (mouse)
- Actin (rabbit)

Secondary antibodies:

- red anti-rabbit IgG (IF)
- red anti-mouse IgG (IF)
- green anti-rabbit IgG (IF)
- green anti-mouse IgG (IF)
- Horseradish Peroxidase (HRP)-conjugated anti-rabbit IgG (WB)
- HRP-conjugated anti-mouse IgG (WB)

Your group will then test your hypothesis by performing an immunofluorescent experiment across the next three weeks. Each of you will individually design a data figure (#1) to report your results. Then your group will test your hypothesis by performing a Western blot experiment across the following six weeks, after which each of you will design a data figure (#2) to report your results.

Table 1. Immunofluorescent (IF) Laboratory Schedule

Date	Activity
Week 1 IF Lab	View live samples on microscope, PFA fix, PBS wash
Week 2 IF Lab	Permeabilize, Block, Add primary antibody
next school day	Remove primary antibody, PBS wash
next school day	Replace PBS wash
next school day	Replace PBS wash
school day before next lab	Replace PBS wash, Add secondary antibody
Week 3 IF lab	PBS wash, Image samples

Data Figure #1: Immunofluorescence Assignment Instructions

Each student individually will need to create a data figure, including figure title and figure legend, that demonstrates your understanding of the lab experiments we did utilizing immunofluorescent techniques.

1) **As you are taking your team's image(s):**

Remember to try to take representative images - those that accurately reflect your team's results. This means you need to look at all embryos and just image one or two/tube.

_____ Record n for each image (number of fish that each image represents out of total number of fish analyzed).
_____ Record total magnification for each image, be sure to include the ocular (eyepiece) lens magnification.

_____ Make note of which direction the head is oriented and which direction the yolk extension is oriented. Ideally the head is pointing to the left and the yolk extension is facing down in each image. You can rotate/flip images later if needed.
_____Record timepoint of each embryo imaged.

2) **Images from all teams will be posted on our learning management system for your use.**

You will need to review all posted images from other groups that tested the same hypothesis as your group and make a conclusion from these combined data. [All images are available for your use as some groups may not have image-able embryos at the end of their experiment.] Then choose the images that you think demonstrate this conclusion best to use in your data figure. In other words, each of you will decide how to organize and display a subset of these images in a figure format that you think best demonstrates the overall results of your experiment.

_____ Be sure to give appropriate credit in your figure legend for images that are not yours.

3) **Include all necessary components for a reader-friendly data figure.**

This means label your images in the data figure as needed! Look at data figures you find in research articles (especially Figure #2 in the "Data Figure Examples Research Article" posted on our learning management system) and include necessary information, symbols, and labels for all images. Some of this information is in the figure itself, and some will be placed in your figure legend – it is up to what you think looks or describe the data best.

_____ These details **may** include: arrows to highlight where readers should pay attention, image 'subtitles,' antibody information i.e. green= what?, which direction each embryo is oriented, magnification.

4) **Write your figure legend:**

Generally - there are two options for legend-writing style: techniques-only vs conclusions-included.

Techniques-only style: Some authors prefer to include only the information necessary for the reader to determine which experiments were done and what data is presented - allowing the reader to then make their own conclusions based on their interpretation of the data.

Conclusions-included: Many authors prefer to include the above information AND additionally describe the conclusion they reached from this data. <u>You will need to write your figure legends in the CONCLUSIONS-INCLUDED style.</u> The trick is to include the necessary technique information, the conclusion AND NOT TOO MUCH EXTRA INFO. Science writing should be succinct.

Make sure to include the following in your figure legend:

_____ which techniques were performed for each sub-figure,
_____ indicate what each fluorophore is staining,
_____ the magnification of each image,
_____ the orientation of the embryo in each image,
_____ make conclusions based on the main question(s) asked – and identify where in the figure the reader can see the data that supports each conclusion. (For example: "In 1A there are positive green nuclei whereas in 1B there are not, demonstrating that _____."),
_____ include other pertinent information you feel is necessary and helpful (see Figure #2 in the "Data Figure Examples Research Article" posted on D2L),
_____ but do NOT include excess information (not a detailed methods section, not a discussion of the results).

5) **Write your figure title:**

_____ This should <u>summarize the overall result(s)</u> displayed in the whole figure (i.e. the conclusion).
_____ For this class, it should NOT be longer than one line of text (12 point, Times New Roman font, 1 inch margins). It should be very short - usually 8-10 words.

Table 2. Western Blot (WB) Laboratory Schedule

Date	Activity
Week 1 WB Lab	Isolate protein from frozen samples
Week 2 WB Lab	Perform BCA protein concentration assay
Week 3 WB Lab	Pour gels, Analyze BCA results, Prepare samples
Week 4 WB Lab	Gel electrophoresis and Transfer to membrane
Week 5 WB Lab	Ponceau stain, Rinse, Block, Prepare primary antibody
school day before next lab	Add primary antibody
Week 6 WB Lab	Rinse membranes, Add secondary antibody, Develop

Data Figure #2: Western Blot Assignment Instructions

Each student individually will need to create a data figure, including figure title and figure legend, that demonstrates your understanding of the lab experiments we did utilizing Western blot techniques.

Images (Ponceau Stain and Western Blot):

_____ indicating which sample was loaded in each lane
_____ label the molecular weight markers and/or label the size of each band
_____ add arrows to the bands of interest, if necessary
_____ indicate loading controls

Figure Legends:

[Same overall description as found in the Data Figure #1: IF assignment.]

_____ make sure to include which techniques were performed for each sub-figure,
_____ indicate what each antibody is staining (i.e. what does pH3 represent? Loading controls?),
_____ make conclusions based on the main questions we were asking as a class – and identify where in the figure they can see the data that supports each conclusion ("In 1A compared to 1B…"),
_____ include other pertinent information (see Figure #5E in the "Data Figure Examples Research Article" posted on D2L for reference),
_____ but do NOT include excess information (not a detailed methods section, not a discussion of the results).

Figure Title:

_____ This should summarize the overall result(s) displayed in the whole figure (i.e. the conclusion).
_____ For this class, it should NOT be longer than one line of text (12 point, Times New Roman font, 1 inch margins). It should be very short - usually 8-10 words.

Notes for the Instructor

Imaging the IF Experiment

Groups rotate through the one or two fluorescent microscopes to image their IF results while the other groups have another assignment they are working on when not imaging. Students use a wide bore Pasteur pipette to transfer ~5 zebrafish embryos at a time onto a depression slide to image. Extra PBS solution is to aid with this transfer.

Safety Concerns

Students need to wear gloves when handling the following reagents: paraformaldehyde (PFA),bis/ acrylamide, TEMED and the chromogenic substrate. The discarded PFA and chromogenic substrate should be collected for proper hazardous waste disposal. The bis/acrylamide is not hazardous after the polyacrylamide gels have solidified, only in liquid form. It is recommended that students wear gloves when handling the IF tubes and the WB equipment, including the membrane so that they do not contaminate the samples.

Helpful Hints

Starting with more embryos than you need for both the IF and the WB always helps. Students appreciate having excess protein sample when it comes time to load the gel for the WB procedure. During the IF procedure, embryos can easily be damaged. We often share successfully stained embryos from one group with less successful groups, so that everyone has an opportunity to image samples.

There are several long incubation times during which our students are working on assignments related to a semester-long group project.

The assessment rubrics found in Appendices E were designed for ease of grading and consistent responses for each student.

We hope you have found useful ideas and exercises to try in your own laboratory course(s). Contact us with any questions. Enjoy your science.

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About the Author

Jenean O'Brien has been an Assistant Professor at the University of Wisconsin Superior since 2015, where she teaches several courses including Genetics, Cell Biology, and Cancer Biology and directs a zebrafish cancer research program for undergraduate students. Jenean loves snow and her Wild family.

Appendix A
Student Instructions: Methods for Immunofluorescence (IF) Experiments

IF Week 1: Microscopy and Preparing Samples for IF Stain

1) Obtain three petri dishes of zebrafish embryos (24 hpf, 48 hpf, and 72 hpf)
2) Observe embryos on the dissecting microscope
3) Use a plastic transfer pipette to remove dead embryos from the dish and discard in waste beaker
4) Label one microfuge tube with ultra-fine tip permanent marker per timepoint needed
    a. All of your tubes should be labeled with your team name initials and the name of the sample
    b. Labels should be directly on the tube (NO TAPE)
    c. Labels should be on the LID and on the SIDE of each tube
    d. Your tube rack should be labeled with your team name on TAPE
5) Use a glass Pasteur pipette with a wide bore to transfer live dechorionated embryos into microfuge tubes:
    a. 20-30 embryos per tube, one tube per timepoint needed
6) Use a glass Pasteur pipette with a narrow bore to remove all the liquid (embryos should remain in tube) from each tube WITHOUT damaging the embryos (having a little liquid left in the tubes is better than harming the embryos trying to get every last drop)
    a. Discard liquid in waste beaker
    b. Retrieve any accidentally discarded embryos with a wide-bore Pasteur pipette
7) Use a transfer pipette and WEAR GLOVES to add ~0.5 ml 4% paraformaldehyde (PFA)/tube to fix embryos
8) Place embryos on the rocker for 1 hour at room temperature
9) Wearing GLOVES, use a narrow bore Pasteur pipette to remove the PFA
    a. Collect PFA waste in the 50 ml conical tube labeled “PFA Waste” for proper disposal
10) Use a transfer pipette to add ~0.5 ml phosphate-buffered saline (PBS) to each tube
11) Rock for 5 minutes
12) Use a narrow-bore Pasteur pipette to remove the PBS (not embryos) and discard in waste beaker
13) Use a transfer pipette to add ~0.5 ml PBS to each tube
14) Rock for 5 minutes
15) Use a narrow-bore Pasteur pipette to remove the PBS (not embryos) and discard in waste beaker
16) Use a transfer pipette to add ~0.5 ml PBS to each tube
17) Store your tube rack at 4°C until lab next week

IF Week 2: Start IF Stain

Note: Watch embryos carefully as they can easily become fragile and fall apart in these washes.

1) Use a narrow-bore Pasteur pipette to remove PBS (not embryos) and discard in waste beaker
2) Use a p1000 pipette to add 200 µl/tube of milliQ-water to permeabilize membranes
    a. Pipette tip can be discarded in regular trash
3) Rock for 5 minutes
4) Use a narrow-bore Pasteur pipette to remove milliQ water (not embryos) and discard in waste beaker
5) Use a p1000 pipette to add 100 µl/tube of IF Block solution
6) Rock for 1 hour at room temperature and do the following during this time:
7) Calculate how many microliters of primary antibody you need in each tube that contains 100 µl total IF Block solution:
    a. pH3 = 1:100
    b. KI67 = 1:20
    c. Et cetera
8) Determine which secondary antibody will go in each of your tubes on the day before lab next week
    a. Write this on the side of each tube
    b. All secondary antibodies will be diluted 1:750 µl IF Block solution
9) Decide which team member will change the solutions in your tube on:
    a. Next weekday at _____: remove primary antibody solution and add PBS wash solution
    b. Next weekday after that at _____: remove PBS wash and add fresh PBS wash
    c. Next weekday after that at_____: remove PBS wash and add fresh PBS wash

   d. Next weekday after that which is also the day before our next lab at ____: remove PBS was and add secondary antibody solution and cover in aluminum foil.
10) After an hour, use a narrow-bore Pasteur pipette to remove IF Block (not embryos) and discard in waste beaker
11) Use a p1000 pipette to add 100 µl/tube of IF Block solution
12) Use a p10 pipette to add your calculated volume of primary antibody to each tube.
13) Store your tube rack at 4°C until lab next week – with one team member coming to perform the tasks listed above each school day from now until then.

IF Week 3: Finish IF Stain and Image

1) Use a narrow-bore Pasteur pipette to remove secondary antibody (not embryos) and discard in waste beaker
2) Use a transfer pipette to add ~0.5 ml phosphate-buffered saline (PBS) to each tube
3) Rock for 5 minutes
4) Use a narrow-bore Pasteur pipette to remove the PBS (not embryos) and discard in waste beaker
5) Use a transfer pipette to add ~0.5 ml PBS to each tube
6) Rock for 5 minutes
7) Use a narrow-bore Pasteur pipette to remove the PBS (not embryos) and discard in waste beaker
8) Use a transfer pipette to add ~0.5 ml PBS to each tube
9) Store your tube rack at 4 °C and in the dark (covered in aluminum foil) until ready to image
10) Rotate groups on the fluorescent microscope to image IF-stained embryos
    a. When it is your group's turn, be sure to follow instructions on Data Figure #1 handout.

Appendix B
Student Instructions: Methods for Western Blot (WB) Experiments

WB Week 1: Lysing Zebrafish Embryos and Protein Concentration Assay

Harvesting Zebrafish Embryos for Western Blot
(already done for you)

1) Dechorionate embryos
2) Transfer 30-50/timepoint into a microfuge tube
3) Incubate on ice for a few minutes
4) Remove embryo media
5) Add 1 ml calcium free Ginzberg Fish Ringers and pipette embryos up and down with a p200 to deyolk the embryos
6) Spin embryos down for 1 minute at low speed
7) Remove Ginzberg Fish Ringers
8) Wash pellet in 1 ml of Deyolking Wash Buffer
9) Spin embryos down for 1 minute at low speed
10) Remove all liquid
11) Place embryos at -80°C and store until ready to use

START HERE Week 1: Lysing Zebrafish Embryos for Western Blot

1) Make a lysis buffer solution with fresh protease inhibitors in an EMPTY microfuge tube:
    a. 200 ul RIPA buffer
    b. 2 ul 100X protease inhibitor cocktail (needs to be added fresh)
2) Add 50 ul lysis buffer solution to each tube of embryos
3) Homogenize with microfuge spear
    a. Use 70% ethanol to rinse spear before using, dry with Kim wipe
    b. Gently grind spear into pellet and buffer
    c. Scrape lysate (as much as possible) off the spear and back into tube
    d. Rinse spear with 70% ethanol before homogenizing next sample
    e. Repeat for each tube
4) Lyse embryos in lysis buffer for 1 hour on ice.
    a. While waiting, label microfuge tubes (Group name & sample name on top & side of tube in permanent marker – no tape) for supernatant (see Step #6)
    b. While waiting, make protein standards for BCA assay (will actually perform BCA assay next week)
5) Centrifuge 15 minutes at 13,000 x *g*
6) Using a micropipette and fresh tips for each sample, transfer the supernatant (~50-60 ul) to a fresh, labeled tube
    a. Discard pellet and old tube
7) Store at -20°C until next week

WB Week 2: BCA Concentration Assay

Perform based on manufacturer's instruction.

WB Week 3: Pour Polyacrylamide Gels, Analyze BCA Assay Results and Prepare Samples

1) Pour correct percent polyacrylamide gel for the size protein you are attempting to visualize, according the vendor's instructions for the equipment your laboratory utilizes
2) The gel will be loaded with each sample in duplicate, where lanes 1-5 are duplicated in lanes 6-10, so that it can be cut in half vertically to create a replicate gel so that two primary antibodies can be utilized per gel.
3) Analyze BCA Assay results to determine how much of each sample you will need in each gel lane
4) Calculate how much Loading Buffer you will need in each gel lane, dependent on buffer concentration and final volume of total sample solution
5) Calculate how much milliQ-water needed in each gel lane to bring each sample solution to the same final volume
6) Thaw samples on ice, thaw Loading Buffer at room temperature
7) Prepare sample solution in a pre-labeled tube for each gel lane (these tubes don't have to be kept on ice)

a. Add ddH2O (don't need to change tips between tubes)
b. Add sample – amount based on BCA Assay results (original samples should be kept on ice, new tip each)
c. Add Loading Buffer – amount dependent of concentration of buffer and final volume of solution (add last because viscous, and because it is viscous be sure you are pipetting the correct amount)

8) Pipette up and down three times to mix sample solution
9) Place tubes in heat block at 95°C for 5 minutes
10) Store samples at 20°C until next week

WB Week 4: Run Gels and Transfer to Membrane

1) Setup gel and load based on vendor's instructions for the equipment your lab utilizes
2) Run gel for 30-40 minutes at 200 volts
3) Transfer gel using a semi-dry transfer system, as the whole process can be completed in 10 minutes total
4) Store membrane in PBS-Tween in petri dish wrapped in Parafilm™ at 4°C until next week

WB Week 5: Block Membrane and Prepare Primary Antibody Solutions

1) Pour off PBS-Tween into sink, keeping your membrane in the petri dish
2) Wearing GLOVES, add Ponceau stain to your dish until you see bands (~2-5 min)
3) Re-collect (pour) Ponceau stain back into the 50 ml conical to reuse
4) Take a picture of your Ponceau-stained blot for your Data Figure. You may need to gently rinse (with deionized water) some of the excess stain off (into the sink) of the blot to get better contrast for imaging. If you wash too much pink stain off, just remove all the water and re-stain in Ponceau (go to #2 above).
5) Rinse membrane with deionized water three times in sink to remove most of the Ponceau stain
6) Pour off all water once rinsed
7) Incubate in 10 ml of WB Block Solution for 1 hour at room temperature on rocker
8) Wash in ~10 ml of PBS-T three time for three minutes each at room temperature on rocker
9) Label and cut your blot into four pieces as diagramed on the whiteboard. [To save on time, each piece will be exposed to a different primary antibody and therefore must include the correct section of the membrane for the target protein's size. Remember you loaded your gel with lanes 1-5 duplicated in lanes 6-10. Each of these halves can then be cut into two pieces, so that one piece is stained with your protein of interest primary antibody and the second piece is stained with a loading control primary antibody.]
10) Store membrane in 10 ml PBS-Tween at 4°C in four Petri dishes wrapped in Parafilm™, each labeled with your full group name and the primary antibody that will be added to it.
11) To prepare primary antibody dilutions for overnight incubation on the day before lab next week, pipette 10 ml of PBS-Tween + 100 µl of WB Blaock into four 15 ml conical tubes, labeled with your full group name and the primary antibody that will be added to it.
12) Add correct primary antibody to each conical:
    A) pH3 – 1:100 so 10 µl
    B) GAPDH – 1:500 so 2 µl
    C) Et cetera
13) Make sure one group member does the following next week on the day before lab:
    a. Pour PBS-Tween off of each piece of membrane from each Petri dish into the sink
    b. Pour primary antibody from each prepared conical onto correct Petri dish.
    c. Wrap Parafilm™ around each Petri dish
    d. Incubate at 4°C overnight

WB Week 6: Rinse Membranes, Incubate in Secondary Antibody, Develop and Image

1) Pour primary antibody back into 15 ml conical tube (can re-use ~3-10 times)
2) Wash for five minutes twice with PBS-Tween (10 ml per Petri dish)
3) Remove last wash and add 10 ml PBS-Tween to each Petri dish
4) Add 3 µl of appropriate HRP-conjugated secondary antibody to each Petri dish
5) Incubate at room temperature on rocker in secondary antibody for 30 minutes
6) Remove secondary antibody and rinse membrane two times briefly with PBS-Tween (enough to cover membrane)

7) Wearing GLOVES, remove PBS-Tween and replace with chromogenic substrate for HRP in each Petri dish
8) Incubate for 30 minutes (or until desired level of sensitivity is attained) on your bench
9) Use a serological pipette to remove the chromogenic substrate and place in the 50 ml conical labeled **Chromogenic Substrate Waste** for proper disposal
10) Wash membrane in milli-Q water for 15 minutes
11) Image and refer to Data Figure #2 handout for instructions.

Appendix C
Materials

This list of equipment and supplies is calculated for the amount needed per group of four students, unless otherwise indicated.

IF Week 1:

Live dechorionated zebrafish embryos at three timepoints:
- 24 hours post fertilization (hpf) embryos (30)
- 48 hpf embryos (30)
- 72 hpf embryos (30)

Petri dishes (3)
Dissecting microscope (1)
Plastic transfer pipette (1)
1.5 ml microfuge tubes (3)
Microfuge tube rack (1)
Ultra-fine tip permanent marker (1)
Labeling tape (1 roll)
Glass Pasteur pipette – wide bore, with rubber bulb (1)
Glass Pasteur pipette – narrow bore, with rubber bulb (1)
Glass beaker for waste (1)
Nitrile gloves
1.5 ml 4% paraformaldehyde (PFA)
50 ml conical for PFA waste (1)
4.5 ml phosphate-buffered saline (PBS)
Rocker (1 per class)
4°C fridge (1 per class)

IF Week 2:

Tubes of embryos in rack from Week 1
Glass Pasteur pipette – wide bore, with rubber bulb (1)
Glass Pasteur pipette – narrow bore, with rubber bulb (1)
Glass beaker for waste (1)
p1000 pipette (1)
p1000 tips (1 box)
600 µl milliQ-water
600 µl IF Block solution
Ultra-fine tip permanent marker (1)
Primary antibodies of interest
p10 (1 per class)
p10 tips (1 box)
Rocker (1 per class)
4°C fridge (1 per class)

Between IF Week 2 and 3:

Tubes of embryos in rack from Week 2
Glass Pasteur pipette – wide bore, with rubber bulb (1)
Glass Pasteur pipette – narrow bore, with rubber bulb (1)
Glass beaker for waste (1)
4.5 ml phosphate-buffered saline (PBS)
p1000 pipette (1)
p1000 tips (1 box)
p10 (1 per class)
p10 tips (1 box)
Fluorophore-conjugated secondary antibodies
300 µl IF Block solution (2% goat serum, 1% bovine serum albumin (BSA), 1% dimethyl sulfoxide (DMSO), 0.1% triton X-100, 1X PBS)
Aluminum foil to cover tube rack

IF Week 3:

Tubes of embryos in rack from Week 2
Glass Pasteur pipette – wide bore, with rubber bulb (1)
Glass Pasteur pipette – narrow bore, with rubber bulb (1)
Glass beaker for waste (1)
10 ml phosphate-buffered saline (PBS, extra for imaging)
Depression slide for imaging (1)
4°C fridge (1 per class)
Fluorescent microscope (1 per class)
Camera/software for fluorescent microscope (1 per class)

Before WB Week 1:

Dechorionated zebrafish embryos at three timepoints:
- 24 hours post fertilization (hpf) embryos (30)
- 48 hpf embryos (30)
- 72 hpf embryos (30)

1.5 ml microfuge tubes (3)
Ice (1 bucket per class)
Glass Pasteur pipette – wide bore, with rubber bulb (1)
Glass Pasteur pipette – narrow bore, with rubber bulb (1)
Glass beaker for waste (1)
3 ml Calcium-free Ginzberg Fish Ringers solution (3.25 g NaCL, 0.125 g KCL, 0.1 g $NaHCO_3$ in 500 ml sterile water)
p1000 pipette (1)

p1000 tips (1 box)
p200 pipette (1)
p200 tips (1 box)
Centrifuge for microfuge tubes (1 per class)
3 ml Deyolking Wash Buffer (11 ml 1M NaCl, 1ml 1M Tris pH8.5, 0.026 g KCL, 0.039 g $CaCl_2$ up to 100 ml sterile water)
-80°C freezer (1 per class)

WB Week 1:
Frozen pellets of 30 zebrafish embryos from above (3)
600 µl RIPA lysis buffer
6 µl 100X Protease Inhibitor Cocktail
Microfuge spear (1)
50 ml 70% ethanol in squirt bottle for rinsing
Kimwipes™
Ice (1 bucket per class)
1.5 ml microfuge tubes (3 + 1 per BCA standard)
Ultra-fine tip permanent marker (1)
200 µg/ml Bovine Serum Albumin (1 ml for standards)
Centrifuge for microfuge tubes (1 per class)
p200 pipette (1)
p200 tips (1 box)
-20°C freezer (1 per class)

WB Week 2:
Samples from last week
Bicinchoninic Acid (BCA) Assay kit
p1000 pipette (1)
p1000 tips (1 box)
p200 pipette (1)
p200 tips (1 box)
p10 (1 per class)
p10 tips (1 box)
1.5 ml microfuge tubes (6 to dilute samples)
milliQ-water (~1 ml to dilute samples)
96 well plate (1)
Aluminum foil to cover 96 well plate
37°C incubator (1 per class)
96 well plate reader (562 nm, 1 per class)
Serological pipette (1 per class to make BCA)
10 ml serological pipette tip (1 per class)
-20°C freezer (1 per class)

WB Week 3:
Gel casting equipment
50 ml 70% ethanol in squirt bottle for rinsing
Kimwipes™
Deionized water in squirt bottle to test for leaks
Nitrile gloves
Moist paper towel and plastic warp to store gel
p1000 pipette (1)
p1000 tips (1 box)
p200 pipette (1)
p200 tips (1 box)
p10 (1 per class)
p10 tips (1 box)
Ultra-fine tip permanent marker (1)
10 ml serological pipette tip (4)
Serological pipette (1 per class)
4°C fridge (1 per class)

For one 12% resolving gel, 1.0 mm thick:
15 ml conical tube (1)
3.4 ml milliQ water
4.0 ml 30% Bis/Acrylamide (NEUROTOXIN)
2.5 ml 1.5M Tris, pH 8.8
100 µl 10% Sodium Dodecyl Sulfate (SDS)
50 µl Ammonium Persulfate (10% APS)
5 µl TEMED (TOXIC)

For one 5% stacking gel, 1.0 mm thick:
15 ml conical tube (1)
1.4 ml milliQ water
400 µl 30% Bis/Acrylamide (NEUROTOXIN)
0.6 ml 0.5M Tris, pH 6.8
50 µl 10% Sodium Dodecyl Sulfate (SDS)
25 µl Ammonium Persulfate (10% APS)
5 µl TEMED (TOXIC)
BCA Assay results from last week
5X Loading Buffer (4 µl per sample)
Samples from last week
milliQ-water (up to 10 µl per sample)
Ice (1 bucket per class)
Heat block for microfuge tubes at 95°C
-20°C freezer (1 per class)

WB Week 4:
Gel running equipment
Transfer equipment and supplies
p20 pipette (1)
p20 gel loading tips (1 box)
10 ml PBS-Tween
Petri dish (1)

Parafilm™

WB Week 5:

Nitrile gloves
20 ml Ponceau stain in 50 ml conical
Cell phone camera
100 ml deionized water in squirt bottle
11 ml WB Block solution (5% non-fat dried milk in PBS-Tween)
110 ml PBS-Tween
10 ml serological pipette tip (4)
Serological Pipette (1 per class)
Rocker (1 per class)
Scissors (1)
Petri dishes (4)
Ultra-fine tip permanent marker (1)
Parafilm™
15 ml conical tubes (4)
Primary Antibodies of interest
4°C fridge (1 per class)

Between WB Week 5 and 6:

Membrane pieces in Petri dishes from last week (4)
Primary antibody solutions from last week (4)
Parafilm™
4°C fridge (1 per class)

WB Week 6:

Membrane pieces in Petri dishes from last week (4)
15 ml conical tube (1 per primary antibody)
200 ml PBS-Tween
3 µl HRP-conjugated secondary antibody/membrane piece
Nitrile gloves
Chromogenic substrate for HRP
50 ml conical for chromogenic substrate waste (1)
10 ml serological pipette tip (1)
Serological pipette (1 per class)
40 ml milliQ-water
Cell phone camera
Rocker (1 per class)

Appendix D
Assessment

Name _____ Score: _____/10 points

Data Figure #1 - IF

The following items were missing (X) or inaccurate (XX):
_____n (number of embryos represented by each image)
_____magnification
_____embryo orientation (which end is anterior?)
_____sample information (zebrafish embryos, 24 hpf, etc.)
_____photo/experimental credit
_____indication of what the reader should focus on (arrows, highlighting, etc.)
_____antigen information – what is GAPDH? pH3? Tubulin? KI67?
_____green/red fluorescence labels

The following items need improvement:
_____too much or inaccurate information in figure legend
_____not enough information in figure legend
_____too much information about methods performed in figure legend
_____techniques performed are missing or inaccurate in figure legend
_____conclusion is missing or inaccurate in figure legend
_____summary of overall result is missing in figure title
_____figure title is too long
_____typos present in figure text
_____disorganized layout or description

Name _____ Score: _____/10 points

Data Figure #2 - WB

The following items were missing (X) or inaccurate (XX):
_____Ponceau stain image
_____indication of which sample was loaded in each lane
_____molecular weight marker identification
_____indication of what to focus on (arrows, highlighting, etc.)
_____loading control information
_____sample information (zebrafish embryos, 24 hpf, etc.)
_____antigen information – what is GAPDH? pH3? Tubulin? KI67?

The following items need improvement:
_____too much or inaccurate information in figure legend
_____not enough information in figure legend
_____too much information about methods performed in figure legend
_____techniques performed are missing or inaccurate in figure legend
_____conclusion is missing or inaccurate in figure legend
_____summary of overall result is missing in figure title
_____figure title is too long
_____typos present in figure text
_____disorganized layout or description

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