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Gene Expression Patterns in *Drosophila* Embryos Using *lacZ* Transgenes

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To appreciate that genes are temporally and spatially expressed, this hands-on laboratory allows students to observe patterns of gene expression in *Drosophila* embryos. Student groups collect embryos containing one of eight *possible* enhancers driving expression of *lacZ*. Subsequently, students run a colorimetric assay to discover the pattern of gene expression which is then documented by microscopy. When the beta-galactosidase protein is expressed from the enhancer-*lacZ* transgene, the location of this enzyme can be detected by the catalysis of a colorless substrate into a bluish-purple colored product. Students submit reports identifying which enhancer drove *lacZ* expression in their unknown transgenic embryo(s).

Keywords: Transgenes, Developmental patterning, Gene expression, *Drosophila*, *lacZ*, Beta-galactosidase, Enhancer, Embryo, Reporter genes

Link to supplementary files: http://www.ableweb.org/volumes/vol-36/silverykey/supplement.htm

Introduction

Objectives

1. To provide students with a hands-on, wet-lab opportunity to investigate gene expression in a multi-cellular organism often used as a model organism for genes affecting human health.
2. To allow students to learn through laboratory application the following genetic terms: t**ransgene, transgenic organism, reporter gene, *lacZ* gene, beta-galactosidase, and enhancer**.
3. To allow students to learn through laboratory application the hierarchy of transcription factors expressed during anterior-posterior (head to tail) axis formation in *Drosophila*: maternal genes, gap genes, segment-polarity genes, pair-rule genes and homeotic genes.
4. Students will learn real-world techniques used in the fields of developmental biology, genetics, and molecular biology, including embryo collection, colorimetric assay and microscopic imaging.
5. Students will observe that the function of the *lacZ* reporter gene is to show a researcher that unique enhancer elements control tissue-specific gene expression.
6. To provide an opportunity for students to analyze data they generate and compare to known samples in order to draw a conclusion about their unknown transgenic *Drosophila* embryos.

Background Information

This lab has been used in a Genetics course for sophomore Biology majors or minors and in upper level Developmental Biology courses. Students must be proficient in using micropipettors and reading detailed procedural lab protocols. For Genetics, the lab occurs after mid-term when students have learned Mendelian Genetics and extensions, plus the Central Dogma of Molecular Biology (Replication, Transcription and Translation). Students are assigned the portion of the Developmental Genetics chapter in their textbooks

(Pierce, 2013), and the PowerPoint presentation on reporter genes and *Drosophila* embryonic development submitted as supplemental materials to the ABLE Proceedings. The lab is related back to the transcription and translation lectures which occur earlier in the semester and then near the end of the semester, gene expression through the lac operon is presented in lecture and students are reminded of the *lacZ* reporter embryo lab at that time. For Developmental Biology, the professors cover the major cell movements and developmental events of *Drosophila* embryogenesis in lecture before the students carry out the lab. While the 25 reviewers suggested that the lab is suitable for all levels of undergraduate courses, the majority of the reviewers recommend this lab for sophomore or junior level students.

The major time consuming step is expanding the eight fly lines starting at least 2.5 weeks before the first day of lab. However, this only involves short periods of instructor time once the flies are in house: specifically, 10 minute intervals twice per week to turn the flies over into new food vials, assuming the Carolina Biological Formula 4-24 food is used. Genesee Scientific Brand fly food (which will require 1 hour to make and aliquot) and embryo collection plates (20 minute procedure) can be made 2-5 weeks prior to the laboratory and kept in the refrigerator until needed. Embryos are collected from flies (at least 50-60 females and 25-30 males) that have been placed in collection cages 2 days prior to lab and 1 overnight collection from each of the fly lines is sufficient for student analysis assuming 1 section of 24 students. Preparation and aliquoting of all solutions can be accomplished within a couple days for initial set-up at an institution and then stored for use in later semesters. Typically, set-up on the day of the lab can be accomplished in an hour or less as long as the solutions have been previously aliquoted. Students can complete the protocol through the staining step in a 1 hour and 50 minute class period (instructors will need to be available for 1 additional hour to stop reactions) and can then spend the next 1 hour 50 minute class session imaging and analyzing their results. ***Note***: During the ABLE 2014 workshop, both sections of faculty were able to complete the entire protocol within the 3 hour allotted time.

Student Outline

Introduction

Frequently scientists analyze the control of gene expression through the use of reporter genes. A reporter gene is like a news reporter. A news reporter goes on location and tells the audience what is going on. Similarly, a reporter gene shows us when and where a particular gene is expressed within a developing organism. By definition, a **reporter gene** is a gene that indicates when and where gene expression is occurring by producing a protein that is easily detectable: either the protein fluoresces or it is an enzyme that can convert a substrate into a colored product. Two attributes characterize a reporter gene. First, the gene is not normally found in the genome of the organism under investigation, and second, it encodes a product that is easily detected.

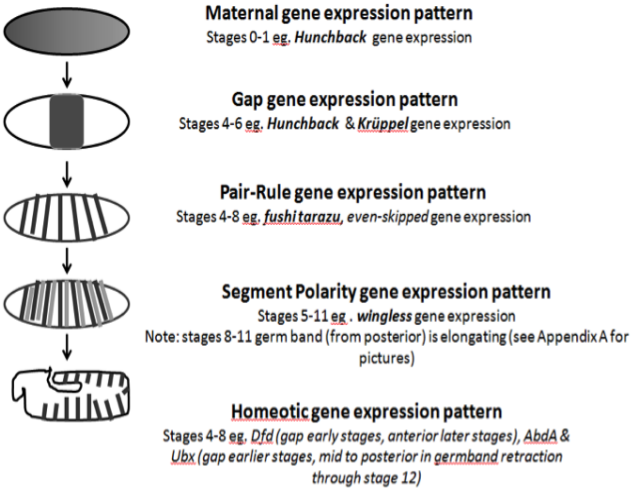

Figure 1. Overview of *Drosophila* embryogenesis indicating at which stage the enhancer-*lacZ* gene expression patterns are expected for each enhancer type (*hunchback, Krüppel, Fushi Tarazu, even-skipped, wingless, deformed, abdominal A* and *ultrabithorax*). While some *lacZ* expression may be seen in most stages of embryogenesis for each transgenic strain, the diagram highlights the embryonic stage most suitable for differentiating between the fly enhancers that drive *lacZ* gene expression in the transgenic unknowns. For accurate depictions of embryos at the various stages, please visit the Flymove and http://insitu.fruitfly.org websites referenced in Appendix A.

For this lab, the b**acterial gene *lacZ*** will be used as a reporter gene in the fruit fly *Drosophila melanogaster.* The *lacZ* gene encodes the **β-galactosidase protein**, which is an enzyme used by bacteria to cleave the β-glycosidic bond found in the disaccharide, lactose. The resulting products are galactose and glucose which the bacterium can use as a source of energy. Conveniently for our purposes, β-galactosidase activity may be detected by providing a colorless substrate with a β-glycosidic bond known as **X-gal** (5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside). When β-galactosidase cleaves the β-glycosidic linkage of X-gal it produces a blue product (5-bromo-4-chloro-indigo). Because *lacZ* is not found in the fly genome, we know that any β-galactosidase expression (i.e. blue staining) results from expression of the *lacZ* reporter gene.

For this exercise you will be using **embryos from transgenic fruit flies**. The Merriam-Webster dictionary defines **transgene** as "a gene that is taken from the genome of one organism and introduced into the genome of another organism by artificial techniques." Therefore, by taking the bacterial gene, *lacZ*, and placing it into the fruit fly genome, *lacZ* is an example of a transgene and the fruit fly containing the bacterial gene is an example of a transgenic organism. Expression is being controlled by the regulatory elements (enhancers and a promoter) that drive the expression of one of the genes in the anterior/posterior (A/P) patterning cascade in *Drosophila*. Each lab group will be supplied with embryos derived from parents that carry an engineered piece of DNA consisting of the *lacZ* gene and the complete regulatory region (one or more enhancer regions and a promoter region) of one of the A/P patterning genes listed in Table 1.

Fruit fly embryogenesis is rapid and occurs within 24 hours (at 25°C) of fertilization. During embryogenesis, the embryo goes through 17 stages as described in Appendix A. Since the embryos for today's experiment have been collected from 0-18 hours after fertilization, every stage shown on the chart in Appendix A may be represented in the embryos observed for this *lacZ* reporter gene lab. It is impossible to predict during what time period the females will lay the majority of their eggs. If a great number of eggs were laid between 4-10 p.m. the night before the lab and embryonic development is stopped by 4°C incubation at 10 a.m. the morning of the lab, then a majority of the embryos will be in the later stages (stages 12-16) of develop-

ment (assuming the flies were reared at 25°C). The details of what the embryo looks like at each of these stages can be found at the Flymove website (see Appendix A for links). In contrast, if the majority of eggs were laid the morning of lab, then the majority of embryos would be 0-6 hours old at stages 1-6 (assuming the flies were reared at 25°C). All of the genes shown in Table 1 below are expressed throughout embryogenesis, but to varying degrees. The maternal affect gene, *hunchback*, will be expressed throughout the entire embryo (ubiquitous expression) in the earliest stages of development (Figure 1, top embryonic gene expression pattern). At stage 0-1, the embryo is one big cell and the *lacZ* pattern should be seen throughout. At stages 4-5, both *hunchback* and *Krüppel* will be expressed in what is known as the 'gap gene pattern'. At this stage, the embryo will have the germ cells (pole cells) located at the posterior of the embryo (see Appendix A). The other gene expression patterns are shown in Figure 1. Please note that *Drosophila* embryos undergo germband extension (stages 8-11) and retraction (stages 12-13) where the posterior extends toward the head and then back towards the posterior. These anatomical markers aid in determining stage. (Please visit the Flymove and *in situ*.fruitfly.org websites as per Appendix A for more details and actual *in situ* embryo gene expression patterns).

You will expose the embryos to **X-gal** to detect *lacZ* expression and then analyze your samples to determine which A/P patterning gene's expression is reflected by the reporter gene. Please note that because of the way these fly stocks are set up 25% of the embryos will **not** carry the reporter construct and will therefore not express *lacZ*. (Please see the Instructor Notes, 'Fly Genetics' section for an explanation). Additionally, the β-galactosidase protein is stable longer than most segmentation proteins so you may see expression at later stages than you expect.

Table 1. *Drosophila* enhancers used to drive the *lacZ* reporter gene.

Enhancer that drives expression of *Drosophila* gene	**Symbol**	**Class of Gene**	**Function**
hunchback	*hb*	maternal effect & gap gene	**Transcription factor (TF)** turns on gap genes
Krüppel	*Kr*	gap gene	**TF** that turns on pair-rule genes
fushi tarazu	*ftz*	pair-rule gene	**TF** that turns on segment polarity genes
even-skipped	*eve*	pair-rule gene	**TF** that turns on segment polarity genes
wingless	*wg*	segment polarity gene	**TF** that turns on homeotic genes
Deformed	*Dfd*	homeotic gene	**TF** that turns on genes to specify body parts
abdominal A	*abdA*	homeotic gene	**TF** that turns on genes to specify body parts
Ultrabithorax	*Ubx*	homeotic gene	**TF** that turns on genes to specify body parts

Learning Objectives

1. Through hands-on participation, students will be able to explain how to collect, fix, and assay *Drosophila* embryos for reporter gene expression.
2. Coupled with a PowerPoint presentation, students will be able to explain abstract concepts:
    a. the terms: **transgene** and **reporter gene** in the context of a living organism.
    b. how different enhancer/promoter regions that drive the reporter gene expression are cell-specific.
3. Students will analyze *lacZ* expression patterns and identify the enhancer/promoter region that is driving transcriptional expression by comparing the patterns to images of known expression patterns.
4. Students will be able to communicate results verbally within and between groups of class peers and in writing through a succinct report of the results and conclusion.

Written Report (See example report, images, and rubric in Appendix C)

This report does not need to be in the standard format for a lab report, but should include the following for each reporter gene line:

1. The plate number of your unknown.
2. Photos of the expression pattern(s) observed indicating which end of the embryo is anterior and which is posterior as well as the approximate stage of the embryo.
3. Name of the corresponding A/P patterning gene.
4. A brief explanation of how you determined the identity of the corresponding A/P patterning gene.

Precautions

- Wear gloves during the entire lab.
- Work in the fume hood when working with solutions containing formaldehyde or heptane to avoid breathing fumes and discard waste in the labeled waste containers.
- The Staining Solution contains compounds conjugated to cyanide. Please use caution when handling this solution and dispose of it in the labeled waste containers.
- Use caution when working with bleach to avoid permanently staining your clothes.
- Keep embryos covered with liquid as much as possible. This means you will need to move rapidly when changing solutions and use volumes indicated.

Procedure

You will collect embryos that are ~0-18 hours old from actively laying cultures, which include large numbers of male and female flies. To collect embryos from these cultures, the flies are kept in bottles capped with a removable Petri dish containing grape juice, sugar, and agar. A dab of yeast paste is placed in the center of each dish as a protein-rich food source. The yeast paste along with the grape odor, attract the females to lay their fertilized eggs on the dish, which can then be removed to collect the eggs.

1. Obtain a bottle of flies. Moving quickly, replace the grape juice-agar Petri dish with a new one containing yeast paste.
2. Label a microcentrifuge tube with your cup number and initials. Add **1 mL water** to the labeled tube.
3. Use a wet paintbrush to collect embryos from the grape juice-agar Petri dish you removed from the bottle, and transfer them to the microcentrifuge tube containing **water** prepared in Step 2. Collect as many embryos as possible but do not exceed the 0.1 mL mark on the tube. Try to avoid getting a lot of yeast which is the brownish paste in the center of the dish, as it can be difficult to remove from the embryos and may interfere with the subsequent staining.
4. Once you have added the embryos to the water, gently invert the tube ~10 times to wash, then put the tube in the rack and allow the embryos settle to the bottom. Remove the water from the tube using a glass Pasteur pipet or a P1000 with appropriate tip. (**Note**: You can use the same glass Pasteur pipet or tip for the entire protocol.) *Some embryos may stick to the side at this point. Just leave them in place and change solutions quickly so they don't dry out.* Repeat this wash by adding another **1 mL water**, invert tube, and allow embryos to settle.
5. Remove water and add **1 mL 50% bleach**. Gently invert the tube ~10 times to mix, and allow the embryos to settle for ~3 minutes. The bleach removes the chorion (the outer eggshell) from the embryos. **It is highly advisable to check that the chorion and dorsal appendages have completely disappeared under the stereoscope before proceeding.** (*Note: Dorsal appendages look like 2 little horns; but are hollow to allow gas exchange between embryo and environment.*)
6. After 2 minutes, remove the bleach (and any embryos that have not settled) and add **1 mL PBS+0.3% Triton**. Gently invert the tube ~10 times, and allow the embryos to settle to the bottom of the tube.
7. Remove the PBS+0.3% Triton, and wash embryos twice more with **1 mL PBS+0.3% Triton** as above. Leave the last wash in the tube until Step 10. (*Note: The 3 washes will remove the bleach to prevent destruction of the embryonic cells.*)
8. Obtain a microcentrifuge tube containing **70 µl 37% Formaldehyde** (premeasured). Take this tube and the tube containing your embryos in PBS+0.3% Triton to the fume hood. (*Note: The formaldehyde will fix the stain and all the molecules within the cell compartments – the molecules and stain will be 'frozen' in position. Avoid inhaling formaldehyde or getting it on your skin.*)
9. While working in the fume hood, add ALL of the **280 µl of Fixative Solution** in the tube to the microcentrifuge tube containing **70 µl 37% Formaldehyde** and gently invert the tube several times to mix. Then add **700 µl Heptane** to the microcentrifuge tube containing the Fixative Solution and Formaldehyde and gently invert the tube several times to mix. USE CAUTION: BOTH HEPTANE AND FORMADEHYDE ARE TOXIC. DO NOT WORK WITH OPEN CONTAINERS OF EITHER HEPTANE OR FORMALDEHYDE OUTSIDE THE FUME HOOD.
10. Continuing to work in the hood, remove the last PBS+0.3% Triton wash from your embryos using a glass Pasteur pipet or a P1000 with appropriate tip and place in the "nonhazardous liquid waste" container. Add ALL of the **Fix/Heptane solution** you prepared in Step 9 to the embryos. Close the lid of the microcentrifuge tube. (*Note: Heptane permeabilizes the vitelline membrane that surrounds the embryo; thus, allowing the beta-galactosidase substrate, X-gal, to enter at Step 17 below.*)

11. Put the microcentrifuge tube containing your embryos on the nutator and allow the embryos to gently rock and fix for 15 minutes at room temperature. Make sure your tube is labeled.

12. While the embryos are fixing, prepare the **X-gal + Staining Solution**.

    a. Obtain microcentrifuge tubes containing **1 mL Staining Solution** and **25 µl X-gal** (8% X-gal in dimethyl formamide) from the 37°C bath. Both have been premeasured for you.

    b. Add all of the **1 mL Staining Solution** directly to the microcentrifuge tube containing the 25 µl of **X-gal**.

    c. Vortex briefly to mix. Pre-incubate **X-gal + Staining Solution** in the 37°C water bath until you are ready to use it in Step 17, but no longer than 2 hours. (*Note: The X-gal will be the substrate for the beta-galactosidase enzyme expressed from the lacZ gene in each transgenic unknown.*)

13. Retrieve your embryos from the nutator and move them to the fume hood. Your tube will contain an aqueous phase and an organic phase. Let the tube sit for a minute or two to allow the phases to separate. The embryos will be at the interface between the two phases.

14. Continuing to work in the hood, remove the **Fix/Heptane** solution from above and below your embryos using a Pasteur pipette and discard into the "Fix/Heptane waste" container in the fume hood. Try to minimize loss of embryos, but realize that you will lose some.

15. Continuing to work in the hood, immediately wash the embryos with **1 mL PBS+0.3%Triton**. Gently invert the tube ~10 times and allow the embryos to settle to the bottom of the tube. Discard this first wash in the "Fix/Heptane waste" container in the fume hood.

16. Take the microcentrifuge tube containing your embryos back to your bench. Wash twice more with **1 mL PBS+0.3%Triton**. The second and third washes can be discarded in the "non-hazardous liquid waste" container on your bench. Using a Pasteur pipet, gently pipet up and down, rinsing the sides of the tubes to dislodge any stuck embryos, and to break up any clumps. (*Note: To prevent making embryonic cells too rigid/fragile, it is important to remove all fix and heptane using 3 washes.*)

17. Remove the final **PBS+0.3%Triton** wash. Add the **X-gal + Staining Solution** prepared in Step 12c to the embryos and mix by inverting gently ~10 times. Incubate your sample in the 37°C water bath. Check the progress of staining at 15 minutes, 30 minutes, 45 minutes and 1 hour, by transferring a few embryos to a slide and examining them using a compound microscope. Make sure you mix the embryos roughly every 15 minutes by inverting gently ~10 times.

18. When the embryos appear sufficiently blue, remove the **Staining Solution** using a glass Pasteur pipet or a P1000 with appropriate tip and place it in the **"Staining Solution Waste"** container. Wash the embryos three times with **1 mL PBS+0.3%Triton**. The first wash should be discarded in the "Staining Solution Waste" container. The remaining washes can be discarded in the "non-hazardous liquid waste" container on your bench. For each wash, add the PBS+0.3% Triton then invert gently ~10 times to mix and allow the embryos to settle. (*Note: It is important to wash off the X-gal 3 times to prevent over staining.*)

19. Place a small drop of embryos in PBS+0.3%Triton on a depression slide and examine using a compound microscope. You will need to **keep a close eye on these embryos while they are on the compound scope** as the heat from the light source will cause the PBS+0.3%Triton to evaporate relatively quickly and you will not be able to rehydrate the embryos once they dry out.

20. Use the digital camera to document the staining pattern of your reporter gene, making sure you can identify which end of the embryo is anterior and which is posterior as well as the approximate stage of each embryo you image.

21. Additional suggestions for scoring your results:

    a. First pass: The broad view

       i. Examine your slide of embryos using either the compound or dissecting light microscope, using magnification that allows you to see a number of embryos at the same time. This broad view will allow you to assess the expression in the population.

       ii. Note that all embryos are not stained, and there should be multiple stages of development, since the embryos that were fixed developed asynchronously.

    b. Second pass: Identify a few representative examples

       i. Identify those embryos that are expressing blue.

ii. Note that the activity of the cis regulatory elements that are driving reporter gene expression may change over time, and since working in isolation from other cis-regulatory regions for that gene, may not illustrate the expression of the corresponding gene at all stages of development. (See Figure, Appendix A and instructor's notes for extra tips on identifying the stages of development; otherwise, use the tips below).

iii. Stage the embryos expressing blue. To stage a *Drosophila* embryo, consider the following suggestions:

1. What is the status of the germ band? Retracted, extended?

2. What is the status of the gut? Is it discernible? Does it have shape? Is the gut heart shaped? That indicates that the embryo is stage 15. Is it puckered into sections, indicating it is stage 16? A clearly discernible tube with loops indicates that the embryo is stage 17.

3. If the embryo is at a stage prior to germ band extension, you can identify the anterior versus posterior end of the embryos by looking for the anterior midgut and posterior midgut invaginations.

4. In fully germ band extended embryos, note that the gut is closer to the posterior than the anterior.

5. Look for the ventral nerve cord, which marks ventral.

c. Third pass: Image a few representative examples

i. Collect images of (at least) two embryos that are representative examples of your mystery gene. If possible, show the mystery gene expression at two stages of development.

Materials

Equipment

- 2-6 stereoscopes (Leica MZ6 or equivalent) and light sources (Fostec gooseneck light)
- Camera on at least one scope (*optional*)
- 1-2 nutators
- At least 1 fume hood for formaldehyde and heptane steps (more are desirable)
- 37°C incubator or water bath
- Wolffe B1-220 compound student microscope (or equivalent) from Carolina Biological Supply for taking digital images of embryos on slides.

Material per Table (for one 4- student group)

The following set up is for a 24-25 student classroom: set up for students working in groups of 4:

- 1 Blue underpad
- 1 Paintbrush
- 1 Non-hazardous waste container
- 1 tube of 50% bleach – (15 mL conical tube with 7 mL bleach and 7 mL water; each student group needs 1 mL 50% bleach)
- 1 conical tube of 1xPBS-0.3%Triton X (10 mL in 15 mL conical tube for each student group)
- 1 P1000 micropipettor
- 1 P200 micropipettor
- 1 box of P1000 tips
- 1 box of P200 tips
- 2 1.5 mL microcentrifuge tubes
- 1 rack (to accommodate the 1.5 mL microcentrifuge tubes)

37°C Incubator or Water Bath

Place the following items in the 37°C incubator or water bath 10 minutes before each section of class begins:

- 25 µL aliquots of 8% X-gal (6, one per student group of 4)
- Aliquots of 1 mL Staining Solution (6, one per student group of 4)
- Rack for incubating embryos that are staining

Instructor's Table

Set up at Instructor's Table

- 6 Grape juice agar plates (one per table) with embryos from unknown transgenic *Drosophila* (enhancer/promoter – *lacZ*)
- Rack of **Premeasured aliquots of 37% Formaldehyde** (70 µL) – ***to be used in hood(s)***
- Rack **premeasured 280 µL aliquots** of Fixative Solution
- Nutator (To rock the embryos during Fixation reaction (15 min)) (*If you don't have access to a nutator, have students hold the bottom of the microcentrifuge tube with their thumb and the top of the microcentrifuge tube with their forefinger and gently rock the tube back and forth so that it is turned completely upside down then right-side up again.*)
- 1 stereoscope (for viewing X-gal-exposed embryos in dissecting dish) and/or 1 Compound microscope (20x objective lens for students to capture images of embryos on slide)

In Hoods

Set up Items in Hood

- Line hood with blue underpad.
- One 50 mL glass bottle of heptane (each student group needs 0.7 mL)
- Labeled Formaldehyde/Fix/Heptane WASTE bottle

Solution Recipes

1.) Fixative Solution

0.1 M PIPES (1,4-Piperazinediethanesulfonic acid disodium salt) pH 6.9
2 mM EGTA pH 8.0
1 mM $MgSO_4$

2.) Phosphate Buffered Saline (PBS)

60 mM K2HPO_4
40 mM KH2PO_4
1.4 M NaCl

3.) Staining Solution

10 mM NaH_2PO_4/Na_2HPO_4 pH 7.2
150 mM NaCl
1 mM $MgCl_2$
3.1 mM $K_4[FeIII(CN)_6]$
3.1 mM $K_3[FeII(CN)_6)]$
0.3% Triton X-100

4.) Grape Juice Agar Plates for Collecting Drosophila Embryos

For 500 mL mix the following 5 ingredients together in a 2L beaker and microwave on high for about 6 minutes, swirling every 2-3 minutes until agar has dissolved:

376 mL water
126 mL grape juice (Welch's concentrate, thawed)
15 g agar
6 g sucrose

Once the agar dissolves, cool for 3 minutes while stirring with a stir bar. Then add the following ingredients and continue stirring:

10 mL 200 proof ethanol
5 mL glacial acetic acid

After mixing, use a 25 mL pipette to aliquot grape juice into 35 mm Petri dishes (Fisherbrand Cat#08-757-11YZ)

5.) Fly Food Options

A. Genesee-Brand Fly food in vials and bottles

- 1 package of Genesee Nutri-Fly mix makes 1 L of food
- Bring 500 mL deionized water to boil in a 1 liter beaker.
- Using a wooden spoon, slowly stir in entire package of food.

Table 2. Sources and suppliers for materials.

A. Supplies		**Catalog#**	**Quantity**	**Company**
Potassium hexacyanoferrate (II) trihydrate ACS reagent, 98.5-102.0% ($K_4Fe(CN)_6$-$3H_2O$		P3289-500g	500g	Sigma-Aldrich
Potassium ferricyanide (III) powder, $K3Fe(CN)_6$		P-8131-500g	500g	Sigma-Aldrich
X-gal (5-Bromo-4-chloro-3-indolyl b-D-galactopyranoside),>98% powder		B4252-2G	2g	Sigma-Aldrich
N,N-Dimethylformamide (DMF) for molecular biology use		D4551-250 mL	250 mL	Sigma-Aldrich
EGTA		E3889-100G	100G	Sigma-Aldrich
PIPES		P1851-500G	500G	Sigma-Aldrich
K_2HPO_4		BP3631-1KG	1KG	Fisher Scientific
KH_2PO_4		P380-500	500g	Fisher Scientific
Magnesium sulfate		MMX0075-1	500g	Fisher Scientific
Fly food options	Nutri-Fly Food (cooking & need mold resistant agents: Tegosept & Proprionic Acid)	66-112	10 (1L) packets/unit	Genesee Scientific
	Tegosept	NC0238407		Fisher Scientific
	Proprionic Acid	ICN15195590		Fisher Scientific
	Formula 4-24 Instant *Drosophila* Medium, Plain (no cooking, contains mold resistant agents, it sets up in 7 minutes)	173200	1L	Carolina Biological Supply Company
Active dry yeast (to sprinkle on top of solidified food - flies mate better with fresh supply of yeast)		62-103	2 lbs/unit	Genesee Scientific or you can buy at grocery stores.
Fly Vials		32-116	500 vials/unit	Genesee Scientific
Flugs® – Fly vial closures (smallest order size) or [cotton balls will work]		49-102 [51-101]	1000 Flugs®/unit [2000 balls/unit]	Genesee Scientific
Fly bottles (to expand stocks for class use, smallest order size)		32-130	500 bottles/unit	Genesee Scientific
Flugs® – Fly bottle closures or [paper lids will work]		49-100 [50-100]	1000 Flugs®/unit [500 lids/unit]	Genesee Scientific
Cheesecloth		53-100	70 yards/unit	Genesee Scientific
Mylar sealing tape (for vials and bottles containing food to be kept in refrigerator)		83-661	216 FT/unit	Genesee Scientific
Embryo dish or depression slide (for viewing staining embryos)		5 0 - 9 3 0 - 3 8 3 S175201	1 each 12/pack	Fisher Scientific
Grape juice agar plates:	35 mm petri dishes (use small side, NOT lid)	08-757-11YZ	500/case	Fisher Scientific
	Welch's frozen grape juice	n/a	340 mL	Any grocery store
	Agar powder	AAA1075236	500g	Fisher Scientific
	200-proof (100%) ethanol	04355451(EA)	gallon	Fisher Scientific
	Glacial acetic acid	BP1185-500	500 mL	Fisher Scientific
	Dextrose (D-Glucose)	D-161	1 kg	Fisher Scientific

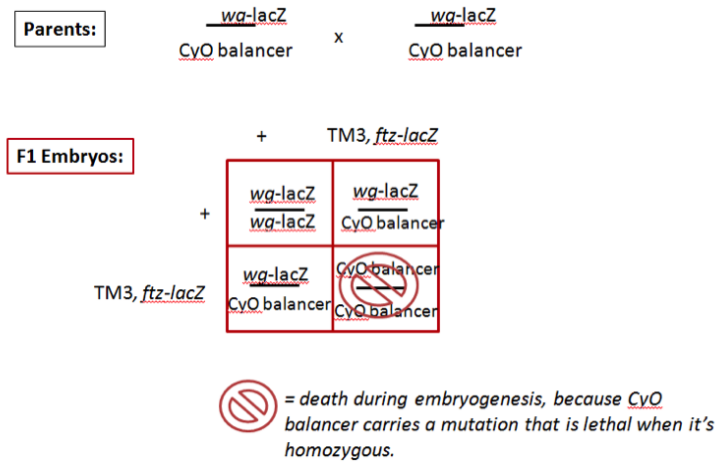

Figure 2. One quarter of the embryos will not carry the *lacZ* reporter gene. This is due to the balancer chromosome, an inverted chromosome used to prevent loss of mutant or recombinant chromosomes in fruit flies. Text within the red boxes in the Punnett Square indicate the predicted embryonic genotypes: 75% should carry at least one copy of the fruit fly enhancer-driven-*lacZ* (bacterial) gene.

- Return to boil and boil 3-5 minutes.
- Reduce heat and cook with stirring (stir bar and occasional spoon) 30 minutes.
- Allow to cool.
- When cooled to 70°C or lower add the anti-fungal agents and stir:
- 0.89g Tegosept (antifungal agent)
- 5mL Propionic Acid (antifungal agent)
- Pipette 25mL into fly bottles or 5 mL into fly vials (See **Table of Sources and Suppliers for Materials** which includes catalog numbers.)
- Cover food containers with cheese cloth for 1-2 days to cure.
- If storing for weeks, then seal food containers with tape and place at 4°C until needed.
- Lids or cotton plugs are placed into bottles or vials, respectively, for use with flies.

B. Formula 4-24 Instant *Drosophila* Medium, Plain from Carolina Biological Supply Company (Item#173200)

- To each vial add 1 tsp Plain Instant *Drosophila* Medium, a dash of instant dry yeast and 5 mL of water. Let sit for 2-3 minutes before adding flies.
- To each bottle add 4.5 tsp Plain Instant *Drosophila* Medium, 2-3 dashes of instant dry yeast and 20 mL of water. Let sit for 2-3 minutes before adding flies.
- Add flies to vial or bottle within 24 hours of adding water.

Drosophila melanogaster stocks routinely used are available for nominal cost from the Bloomington Drosophila Stock Center (BDSC), Bloomington, IN:

a. BDSC#76: Dmel\P{Thb8-lacZ}WD1,TM3 (aka ***hb-lacZ***), example of maternal effect/gap gene

b. BDSC#335: Dmel\P{eve-lacZ8.0}SB1, SM6b (aka ***eve-lacZ***), example of pair-rule gene

c. BDSC#1672: Dmel\Sna(Sco)/CyO, P{en1}wg^en11 (aka ***wg-lacZ***), example of segment polarity gene

d. BDSC#1671: Dmel\red{1}e{1}mbc[C1]TM3, P{ry{+t7.2]=**ftz-*lacZ***.ry[+]}TM3, Sb[1]ry[*], another example of a pair rule gene.

e. BDSC#10392: Dmel\P{lacW}Kr^k05826(aka ***Kr-lacZ***), example of gap gene

f. BDSC#9120: Dmel\P{**Ubx-*lacZ***.w+}TM3 (aka ***Ubx-lacZ***), example of homoeotic gene

C. Supplier URL Addresses:
Sigma-Aldrich:
http://www.sigmaaldrich.com/sigma-aldrich/home.html
Fisher Scientific:
http://www.fishersci.com/ecomm/servlet/cmstatic
Genesee Scientific https://geneseesci.com/
Bloomington Stock Center:
http://flystocks.bio.indiana.edu/

Notes for the Instructor

Introduction

A supplementary PowerPoint Presentation from the ABLE 2014 meeting at the University of Oregon at Eugene should help introduce the lab and concepts. Additionally, three supplementary videos have been provided: Two showing fly transfer technique to maintain and expand flies and one showing the collection of embryo-containing eggs on a grape juice agar plate.

ABLE Faculty Requests

A few of the faculty that attended this major workshop requested ideas for expanding this laboratory making it more inquiry-based or discovery-based. The authors acknowledge that this could be accomplished. One idea from a peer reviewer was to have a semester-long project on transgenes. Another peer reviewer suggested that the lab could be followed up with bioinformatic research questions, perhaps focused on investigation of the *Drosophila* genes that are normally driven by the enhancers showcased in this lab: species or organism comparisons.

Fly Genetics

The transgenic flies are created by inserting a DNA construct derived from a transposable element (Bachmann and Knust, 2008) such that it contains the enhancer-*lacZ* recombinant and then maintained in the fly genome by using balancer chromosomes (Roote and Prokop, 2013). Balancer chromosomes cannot recombine with the homologous chromosome, which has the enhancer-*lacZ* gene, because the order of the genes has been shuffled in a balancer chromosome: Therefore, the loci no longer line up, which prevents homologous recombination and the potential loss of mutant or recombinant chromosomes in fruit fly strains.

The balancer chromosome for chromosome #3 is typically TM3 and carries a visible marker: either *Sb* (*Stubble* (short) thoracic bristles) or *Ser* (*Serrate* wing-notch phenotype). While, the balancer chromosome, CyO or SM6b, for chromosome #2 usually carries the visible marker Cy that results in curly wings. Additionally, the CyO balancer carries a mutation that is lethal in homozygous embryos.

The embryo will be present, but will not mature beyond embryogenesis. The lethal allele strategy allows the fly stock to maintain the heterozygous *lacZ*/balancer flies. Therefore, because the transgenic fly strains are heterozygous, then 25% of the embryos will be homozygous for the non-*lacZ* chromosome. See Figure 2 for the Punnet Square of the *wingless* (*wg*)-*lacZ* example.

Fly Maintenance

Healthy, well-fed flies must acclimate to the embryo collection cages (see below section on embryo fly cages) at constant temperature for 24 hours before egg collections can be made. Flies cannot be frozen, they must be maintained year round. Typically, the flies are kept in 2 sets of vials with 10 mL food per vial and stopped with a cotton plug. For vials kept at room temperature adult flies should be transferred/turned over into a new vial with fresh food at least 1x per month keeping one vial per set with pupal cases until you are sure that the newly seeded vial has feeding larva churning up the food. (See supplementary videos for fly transfer technique.) To transfer adults into a new vial, tap the vial that contains the adult flies onto a counter top so that the flies are knocked down to the bottom of the vial. Remove the cotton plug from this vial and quickly align the openings of the vial containing the flies with the new vial. Tap the aligned vials on the countertop until the adult flies fall into the new vial. Remove the old vial and continue tapping the new one to prevent the adult flies from climbing out, then plug the opening of the new vial with a fresh cotton ball or Flug® closure and label the vial with the genotype of the stock. We recommend viewing one of the several videos available on YouTube that demonstrate this process before you try it for the first time. The fly life cycle from embryo to adult is 10-14 days. The lifespan can be extended by housing the fly stocks at 18°C; which roughly double their developmental cycle.

Preparation Notes

Allow at least 2.5 weeks for transgenic *Drosophila* strains to expand in bottles when starting from stock vials. However, this only involves short periods of instructor time once the flies are in house: Specifically, 10 minute intervals twice per week to turn the flies over into new food vials (supplemental video clips provided for turnover/transfer technique), assuming the Carolina Blue food is used. Genesee Scientific Brand fly food (which will require 1 hour to make and aliquot) and embryo collection plates (20 minute procedure) can be made 2-5 weeks prior to the laboratory and kept in the refrigerator until needed. Genesee-Brand Fly food and grape juice agar plates can be made up to a month in advance of the lab and then when sealed, both will store at 4°C for a month.

The X-gal and 1mL aliquots of staining solution can be made in advance and stored at -20°C for at least a year. Fix and PBS solutions can be made in advance and stored at room temperature indefinitely.

As the de-chorionation (eggshell removal) step is essential for the procedure to work, the 50% BLEACH solution must be made fresh (within the week). It is highly recommended that instructors test the bleach solution prior to student use by simply exposing embryos to bleach and observing the successful removal of the chorion. *Drosophila melanogaster* eggs have two dorsal appendages. (These look like horns, but are hollow tubes to allow gas exchange between embryo and outer environment.) You will observe with a stereoscope that the dorsal appendages dissolve within 3 minutes of exposure to 50% bleach.

Fly Cage Setup for Embryo Collection

(Note: See supplementary videos for 'Collection of Embryos' technique.) Embryos are collected using a fly cage, which consists of a plastic fly bottle with a cotton ball plugged gas exchange hole that is capped with a grape juice agar plate. To make the gas exchange hole, heat a metal dissecting probe or needle using a Bunsen burner and use it to cut a dime sized hole in the side of a plastic fly bottle. Plug this hole with a fresh cotton ball. Make yeast paste by mixing active dry yeast with water so that you get a paste that has the consistency of creamy peanut butter. Place a pea-sized dab of yeast paste onto the center of a grape juice agar plate. Transfer ~100 adult flies into the fly cage using

the tapping method described above. Cap the fly cage with the grape juice agar plate + yeast paste, using lab tape to hold the plate in place. Invert the fly cage so that the food source (i.e. the yeast paste) is on the bottom. The female flies will be attracted to the yeast paste and the smell of the grape juice and will lay their eggs on the grape juice agar plate. When you are ready to collect the embryos that have been laid on the plate, loosen the tape around the plate, tap fly cage on countertop so the adult flies fall to the bottom, remove the old plate and replace it with a fresh grape juice agar plate + yeast paste.

Day of Lab

Pre-warming the X-gal and staining solution at 37°C is necessary to allow visualization within the time frame of lab class. At NCCU, the lab time is only 1 hour and 50 minutes long and it is often necessary to have instructor finish staining and students view the stained embryos during the next class period.

Visualizing Embryos

A stereoscope with 40-60x magnification is sufficient for viewing staining embryos. However, for photographing embryos, mounting the embryos on a slide in 70% glycerol and viewing on compound scope at 100x works well for photography. Use double sided tape to prevent crushing the embryos and seal the slide with clear nail polish.

For a given enhancer-*lacZ* transgenic embryo strain, the pattern of gene expression will vary depending on the developmental stage the embryo is in. Therefore, to identify those samples in which the reporter gene expression is driven in a pattern most reflective of the corresponding gene, consider the following suggestions:

1. *hb-lacZ*: expressed early, before germ band extension
2. *Kr-lacZ*: expressed early, before germ band extension
3. *eve-lacZ*: expressed both before, during, and after germ band extension
4. *wg-lacZ*: expressed during and after germ band extension
5. *Ubx-lacZ*: expressed during and after germ band extension

- Please visit the following website for http://flymove.uni-muenster.de/ and click on the 'Stages' tab to explore stages of *Drosophila* embryogenesis. The time laps move "Embryogenesis, in vivo" is especially informative in illustrating germ band lengthening (extension/elongation, stages 8-10) and germ band shortening (retraction, stage 12) as well as when segmentation and midgut formation.
- For an overview of the *Drosophila* A/P patterning cascade and descriptions of the roles of the segmentation genes mentioned in this exercise, please see Gilbert (2013) or visit the following website http://flymove.uni-muenster.de and click on "Processes" and then "Segmentation".

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Literature Cited

Bachmann, A. and E. Knust. 2008. The use of P-element transposons to generate transgenic flies. *Methods Mol Biol*. 420:61-77.

Gilbert, S. F. 2013. *Developmental Biology*. Sunderland, MA: Sinauer Associates. 750 p.

Perrimon, N., E. Noll, K. McCall, A. Brand. 1991. Generating lineage-specific markers to study *Drosophila* development. *Dev Genet*. 12(3):238-52.

Pierce, B.A. 2013. *Genetics: A Conceptual Approach*. New York, NY: W.H. Freeman. 700 p.

Roote, J. and A. Prokop. 2013. How to Design a Genetic Mating Scheme: A Basic Training Package for *Drosophila. Genetics*. *G3*. 3:353-358.

About the Authors

Dr. S. Catherine Silver Key is an Associate Professor of Biology at North Carolina Central University. She received her B.S. in Biology from the University of Missouri at St. Louis and her Ph.D. in Microbiology and Immunology from University of North Carolina at Chapel Hill. She teaches undergraduate Genetics (BIOL3100), Cell and Molecular Biology (BIOL2200), Introduction to Research (BIOL4400), Inquiries in Developmental Biology (BIOL4100), and Graduate Genetics (BIOD8020/BIOG5120). She also mentors undergraduates in BIOL4400 and from various training grants (RISE, MARC, FUTURES, S-STEM) and graduate students at the NCCU Master's Program in her *Drosophila*-based research lab focusing on the effect of the CRL4Cdt2 ubiquitin-mediating complex during development and *Drosophila* genome annotation through the Genomics Education Partnership (GEP, Dr. Sarah Elgin, P.I.). Dr. Silver Key began implementing the *Drosophila* embryo enhancer-*lacZ* lab in Spring 2013 after receiving funds for implementation from the Roberta Williams Teaching Initiative Grant through the Association for Biology Laboratory Education.

Dr. Julie Gates is an Associate Professor of Biology at Bucknell University. She received a B.S. in Molecular Biology from the University of Wisconsin and a Ph.D. in Human Genetics from the University of Utah. She teaches Biology for Non-majors (BIOL 121), Lab for Introduction to Mol-

ecules and Cells (BIOL 205L), and Developmental Biology (BIOL 339). She started using the *Drosophila* embryo enhancer-*lacZ* lab in her Developmental Biology course in Spring 2007 and it has become a regular component of this course each spring semester. She also regularly mentors undergraduate research students during the academic year as well as over the summer. Her research focuses on how modification of the actin cytoskeleton allows cells to move and change shape as tissues and organs are formed in the *Drosophila* embryo using a combination of genetic, cell biological and molecular techniques. In May 2012, she received the William Pierce Boger, Jr., M.D. Award for Excellence in Teaching in the Natural Sciences from Bucknell University.

Dr. Jessica K. Sawyer is a postdoctoral fellow at Duke University. She received a B.S. in Biology from Virginia Tech and a Ph.D. in Biology and Developmental Biology from the University of North Carolina at Chapel Hill. She is currently in the laboratory of Dr. Don Fox and conducts research about factors that impact tumorigenesis using *Drosophila* as a model. She is a Preparing Future Faculty (PFF) fellow and was mentored by Dr. S. Catherine Silver Key for the 2013-2014 academic year. She assisted and taught the *Drosophila* expression laboratory in Spring 2014. She also mentors undergraduates at Duke.

Dr. Kirsten A. Guss is Associate Professor of Biology and the John R. Stafford and Inge Paul Stafford Endowed Chair in Bioinformatics at Dickinson College. She is also a member of the Biochemistry & Molecular Biology Program. She teaches at all levels in the undergraduate biology curriculum: This is Your Life (an overview of the human life cycle, BIOL127), Genetics (BIOL216), Animal Development (BIOL318) and Developmental Genomics (BIOL418). Her research focuses on the transcriptional control of gene expression during *Drosophila* development. She designed the *Drosophila* embryo *lacZ* gene expression lab while she was a postdoctoral fellow at University of Wisconsin-Madison, to accompany lectures given by her mentor Sean B. Carroll. She continues to employ the protocol in her Animal Development class at Dickinson, with ten successful implementations to date.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/**

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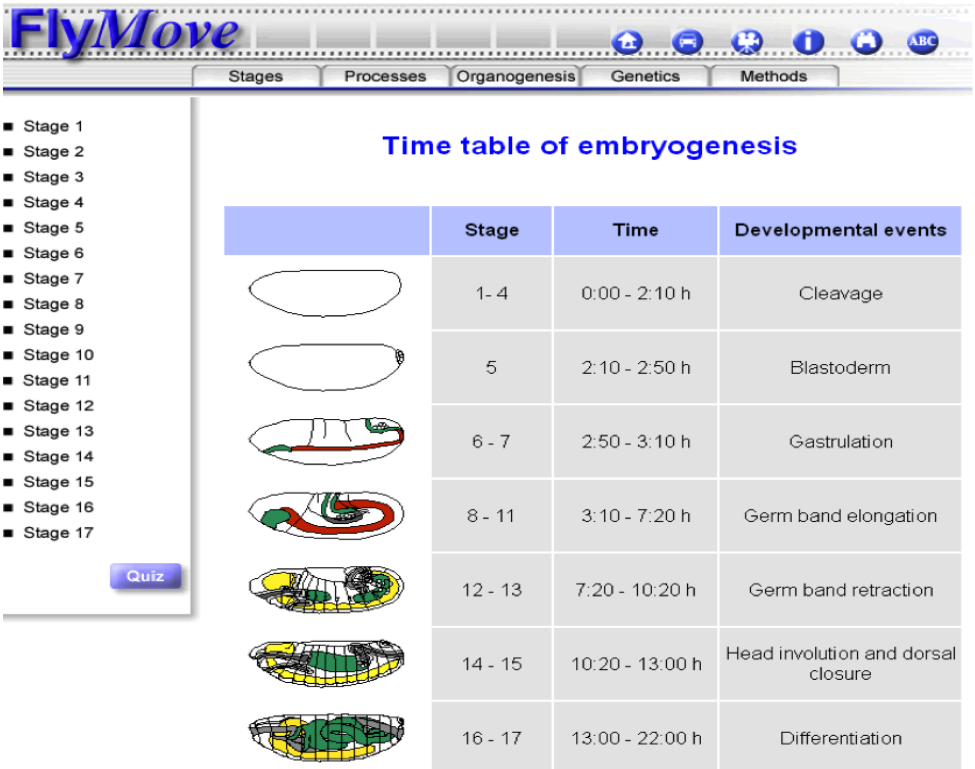
Appendix A
Reference Images for Gene Expression Patterns

Embryos that students are observing range in age. For *Drosophila* embryos the age can be denoted as 'stage'. As shown in Figure 3 (from FlyMove at http://flymove.uni-muenster.de/), embryos collected for varying amounts of time are at different stages. The enhancer-*lacZ* embryos were collected overnight or 0-18 hours which would be stages 1- ~16.5.



	Stage	Time	Developmental events
	1- 4	0:00 - 2:10 h	Cleavage
	5	2:10 - 2:50 h	Blastoderm
	6 - 7	2:50 - 3:10 h	Gastrulation
	8 - 11	3:10 - 7:20 h	Germ band elongation
	12 - 13	7:20 - 10:20 h	Germ band retraction
	14 - 15	10:20 - 13:00 h	Head involution and dorsal closure
	16 - 17	13:00 - 22:00 h	Differentiation

Figure 3. *Drosophila* embryonic stages as described on FlyMove.

When trying to figure out which enhancer-*lacZ* embryo we have we can use a reference. The reference we will use is the embryo images cataloged at the Berkeley *Drosophila* Genome Project website: http://insitu.fruitfly.org/, there is a search engine on the home page if the sites listed below become archaic. At this website the gene expression patterns are shown by using a technique known as *in situ* hybridization. In this technique, scientists make a piece of RNA that has a detectable label on it and then use temperature changes to allow complimentary base-pairing of the labeled-RNA to the mRNA expressed in the cells of the embryo. In contrast, you are looking at the gene expression pattern using the *lacZ* reporter gene. Nonetheless, the pattern is the same and you can use the patterns shown in the online database as a reference to figure out your 'unknown' gene expressed in your embryo.

Example Patterns in Reference Embryos to Use in Determining the Identity of Your Unknown Gene: Only one representative picture of each gene's expression pattern is shown below. *Look at several different stages to make an accurate conclusion about the identity of the gene* being expressed in your 'unknown' transgenic embryo.

abd-A = *abdominal A*
Example shown is stage 11-12
For more stages of *abd-A* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=3&ftext=PC00111

Anterior  Posterior

Dfd = *Deformed*
Example shown is stage 11-12
For more stages of *Dfd* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=3&ftext=IPI4630

Anterior  Posterior

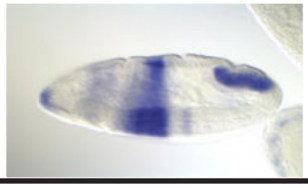
eve = *even skipped*
Example shown is stage 7-8
For more stages of *eve* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=3&ftext=RT01063

Anterior  Posterior

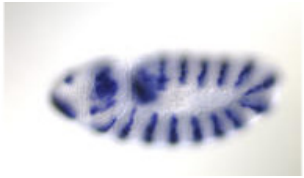
ftz = *fushi tarazu*
Example shown is stage 11-12
For more stages of *ftz* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=3&ftext=CG2047

Anterior  Posterior

Kr = *Krüppel*
Example shown is stage 4-6
For more stages of *Kr* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=3&ftext=RE30918

Anterior  Posterior

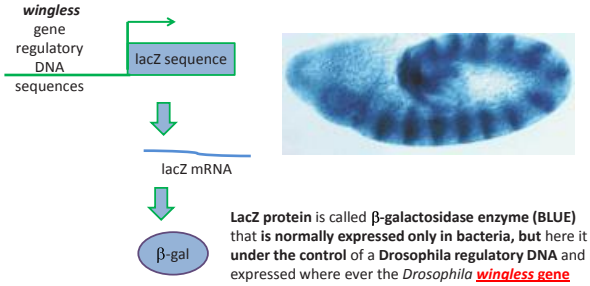
wg = *wingless*
Example shown is stage 11-12
For more stages of *wg* go to:
http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=1&ftext=CG4889

Anterior  Posterior

Appendix B

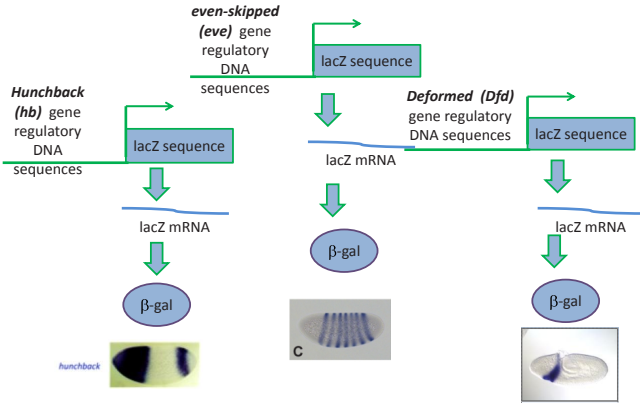
Example Slides Used to Explain Concept of *LacZ* Reporter Gene in Transgenic Animal

Bacterial *lacZ* Gene Reports Where Fly Genes are expressed in an embryo



LacZ protein is called **β-galactosidase enzyme (BLUE)** that **is normally expressed only in bacteria, but** here it is **under the control** of a **Drosophila regulatory DNA** and is expressed where ever the *Drosophila* ***wingless* gene** would **normally be expressed** in the ***Drosophila* embryo. The blue color 'reports' to us that the lacZ gene has been expressed in the cells that *wingless* would normally be expressed in.**

Different *Drosophila* regulatory sequences can turn on the bacterial lacZ reporter



Transgene & transgenic organism

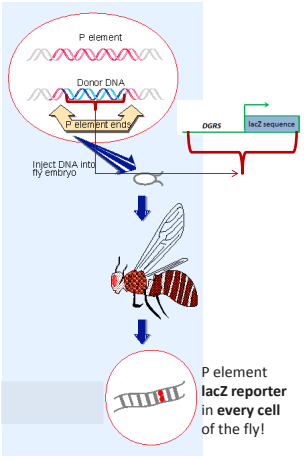
Study about 'Transgenic Animals' on pp 542-543 in Pierce 4e Text

HOW DID THE BACTERIAL LACZ GENE SEQUENCE GET INTO THE *DROSOPHILA* GENOME?

Making a Transgenic Fly

- P element = transposable element
  - Which can insert into the fly genome
- The P element genes in the middle are removed
- And replaced by Donor DNA
- Donor DNA =

***D*rosophila *G*ene *R*egulatory *S*equences(DGRS)** lacZ reporter gene sequence



P element **lacZ reporter** in **every cell** of the fly!

Appendix C
Example Student Data (Written Report/Rubric/Images)

Sample Student Report
Student Name
BIOL 3100-01
Cup #7

Introduction:

Drosophila gene expression plays a key role in how a fly is shaped. There are three different types of genes that control each stage of development in fruit flies. There are the egg-polarity genes which control the establishment of main body axes, the segmentation genes which determine the number and polarity of body segments and the homeotic genes which controls the establishment of identity of each segment. Each of these genes encode for different transcription factors which turn on gene expression.

For this experiment we were given an unknown embryo containing eggs that were laid by different transgenic flies. We wanted to figure out which *Drosophila* regulatory elements turned on the lacZ gene. We removed the chorion from the embryos by bleaching them. Then we fixed the embryos. We were then given beta-galactosidase enzyme (from the lacZ reporter gene) a colorless substrate and developed a blue pattern (staining). We allow the embryos to sit until after they were blue. After they were we observed the embryos to see which transcription factor pattern was present and responsible for turning on gene expression.

Results:

After observing our embryo we conclude that the wingless patterning gene was responsible for this embryo. The embryo had to be in the segmentation developmental stage. In the picture below you can see the anterior region at the top left of the picture and the posterior region at the bottom right of the picture. It appears to me that the embryo seems to be ready to move to next stage of development. The head, thoracic segments, and abdominal segments all seem to be almost fully developed. The margins/segments are very visible.

Conclusion:

To identify the corresponding a/p patterning gene I closely observed the pattern visible in our embryo and compared it to the different patterning genes. I initially thought that the patterning gene present was the *even-skipped* patterning gene. After a closer observation of the margins or segment lines I determined that this was actually the wingless patterning gene. The *wingless* patterning gene is more defined than that of the *even-skipped* patterning gene. The ratio of the head, to thoracic, to abdominal segments also seems more close in the wingless pattern that the even-skipped pattern to me.

***Drosophila* Gene Expression Report Guidelines and Evaluation Rubric**
BIOL3100

Your assignment – you will be graded on the following criteria:

1. Lab Group Information:
- Student Name
- Course Number and Section
- Number of Cup used for the experiment
- Group Members

2. Introduction:
- Write a brief summary of the purpose of the experiment.
  - What are you trying to identify?
  - What is the reporter gene you are using to visualize the gene expression pattern?

3. Results:

- Include a photo of the expression pattern observed
  - Indicate which end of the embryo is anterior and posterior
  - Include approximate stage of embryo
  - Include name of the corresponding A/P patterning gene
- Write a brief summary of the pattern observed.

4. Conclusions:

- Write a brief explanation of how you determined the identity of the corresponding A/P patterning gene.

TOTAL LENGTH: NO MORE THAN 1 PAGE
Assignment total point value: 30 points.

Required Graded Element	Max Point Potential	Points Earned
Lab Group Information: Include all information detailed above.	4	
Introduction: Briefly describe the purpose of the laboratory.	6	
Results: Include photo. Label the photo indicating anterior/posterior, stage, and name of A/P patterning gene. Include a brief summary of the pattern your observed.	10	
Conclusions: Write a brief summary of how you identified the A/P patterning gene.	10	
TOTAL		30

Comments:

***Drosophila* Expression Lab Images**

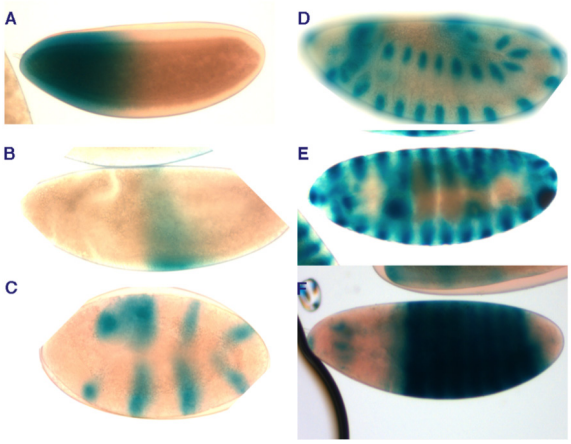


Figure 4. Typical student data generated using this protocol.

Images were collected using a Nikon E800 compound microscope equipped for differential interference contrast illumination. Images were cropped and adjusted for brightness, contrast, and levels using Adobe Photoshop. All embryos are oriented anterior to the left, and were staged according to Hartenstein, 1993. A, BDSC#76 (*hb-lacZ*) shows intense staining in the anterior end of a stage 5 embryo. B, BDSC#10392 (*Kr-lacZ*) shows a stripe of expression about mid-length of a stage 6 embryo. C, BDSC#335 (*eve-lacZ*) shows seven stripes of expression in a germ band extended (approximately stage 10) embryo. D, E, BDSC#1672 (w*g-lacZ*) shows stripes of expression along the length of stage 12 (D) and stage 15 (E) embryos. F, BDSC#9120 (*Ubx-lacZ*) shows expression a subset of thoracic and abdominal segments.

Less sophisticated imaging can also provide students with usable data (cell phone images and bright field microscopy):

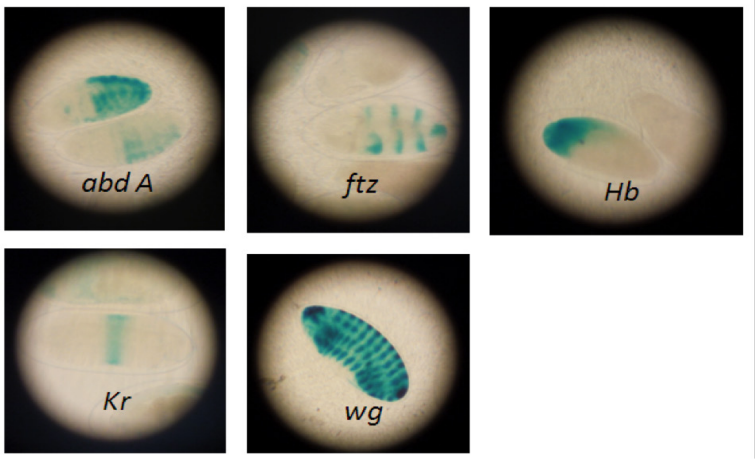


Figure 5. Example of stained transgenic embryo images from students at North Carolina Central University. Whole enhancer-*lacZ* embryos were mounted slides on slides. *Top row embryos left to right: abdominal A (abdA)-lacZ, fushi tarazu (ftz)-lacZ, hunchback (hb)-lacZ. Bottom row embryos left to right: Krüppel (Kr)-lacZ* and *wingless (wg)-lacZ.*

Slide Preparation and Photography:

First, the embryos in 1xPBS were pipetted onto slides(frosted labeling slides). The PBS was removed by pipetting and replaced with ~20µL of 70% glycerol. Embryos were gently separated using a pipette tip. To prevent embryos from being squished, double-sided tape was placed to the left and the right of the embryo-containing glycerol area. Then the cover slip was applied. Finally, the cover slip was secured to the slide by painting clear nail polish around the edges of the cover slip. Images were taken with digital camera phones using a bright field compound microscope (Wolffe B1-220, Carolina Biological Supply Co.) set at 200x. (Images could also be captured with Nikon FinePix digital camera using the Orion 5338 SteadyPix Deluxe Camera Mount (available through http://www.amazon.com).

APPENDIX D
Sample Student Reaction to Lab (Student Comments/Student Learning Gains)

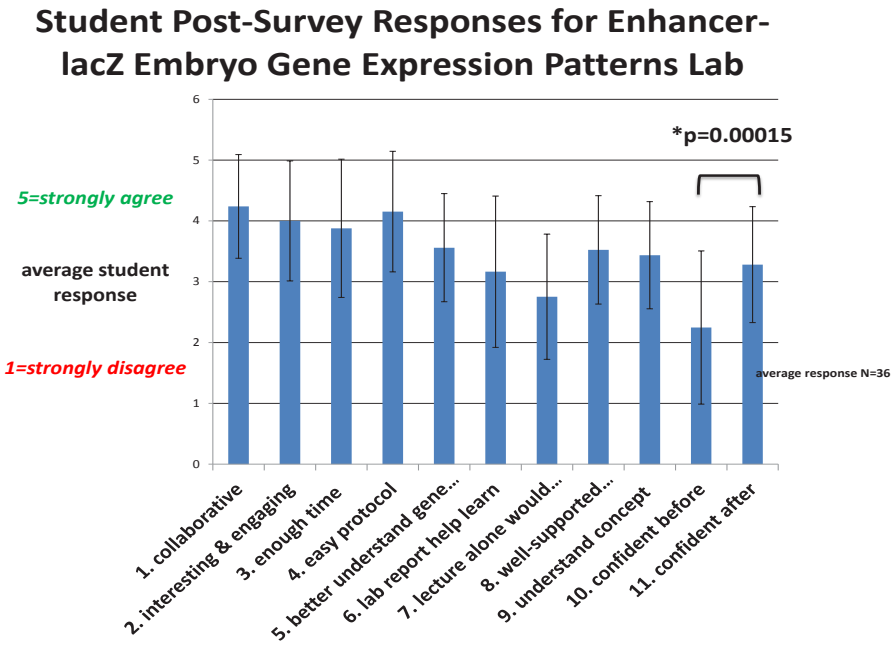
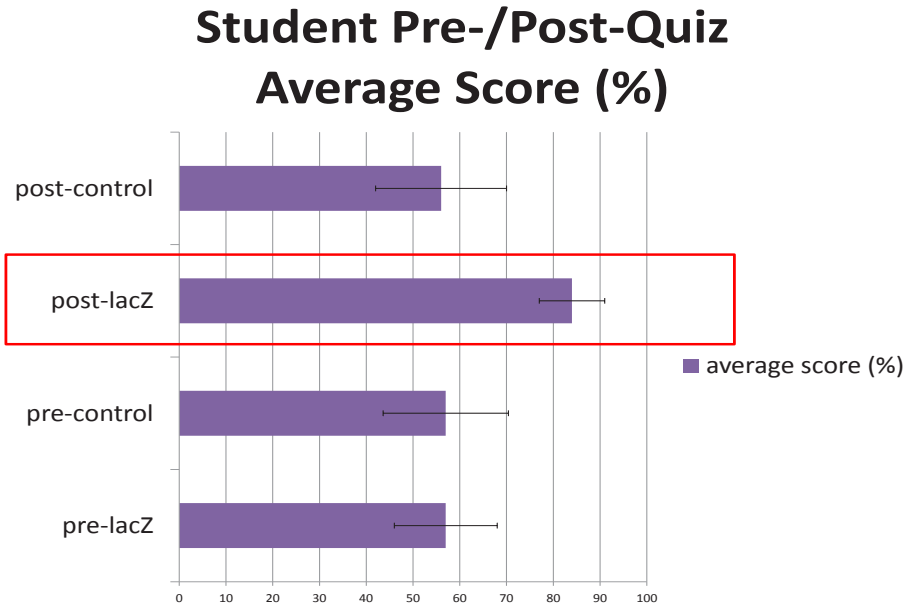


Figure 6. Student Post-Survey for Enhancer-*lacZ* Embryo Gene Expression Patterns Lab. Spring 2014 BIOL3100 (Genetics) North Carolina Central University students were asked to respond to statements after participating in the lab on whether the agreed with each statement on a scale of 1-5 with 1=strongly disagree and 5=strongly agree. In this one semester run, surveying 36 students after participating in the lab activity, the results suggest that student confidence in understanding the concept of gene expression in eukaryotes has significantly increase (p= 0.00015 when comparing responses to statements 10 and 11). Students also responded in agreement to statements that the lab was 1.collaborative (4.2±0.9), 2.interesting & engaging (4.0±1), 3. Enough time to complete lab (3.9±1), 4. Easy protocol (4.2±1), and 5. Better understanding of gene expression concept (3.6±0.9). Notably, students were either neutral or disagreed with the statement that the lecture alone would suffice in understanding the concept of gene regulation (2.75±1).



BIOL 339 (Bucknell University) Post-Survey Written Responses - Spring 2013

What do you think is beneficial about learning using this particular activity?

- It's hands-on and gave us the opportunity to apply lecture to lab to better understand the material.
- I think any hands on lab work is beneficial overall in better understanding what is happening and what has been discussed in lecture. I found it to be helpful in comparing different gene expressions.
- It helps to supplement material covered in lecture.
- Getting a visual representation of gene patterning and seeing how they look in 3D embryos, not just textbook drawings.
- You were able to visualize where the different genes were expressed.
- It is a hands on activity plus you get to see exactly how things you learn about in lecture really work.
- Use of tangible experiment to see what we lecture about in class.
- It gave an applicable situation that was able to involve the lecture material and helped me understand it better.

What did you learn from using this activity?

- The expression of different genes at different stages as well as how to visualize and use the technique of a reporter gene, specifically *lacZ*.
- I got a better understanding of how these genes were really expressed and was able to compare them easily too.
- More about Drosophila development; reporter genes and A/P patterning.
- Identifying embryo stages, staining procedure, applying patterning material from class to determine the type.
- Not much in addition from lecture. It just clarified the concepts. How to use a reporter gene in lab.
- I learned how Drosophila start to develop and what actions they must go through.
- Different stages of embryonic development. Different patterns of expression.
- I learned the different phases of development much more thoroughly (specifically the different cell types and their movements as development progressed).