The Power of Drosophila Genetics

Biol 486H Spring 2016

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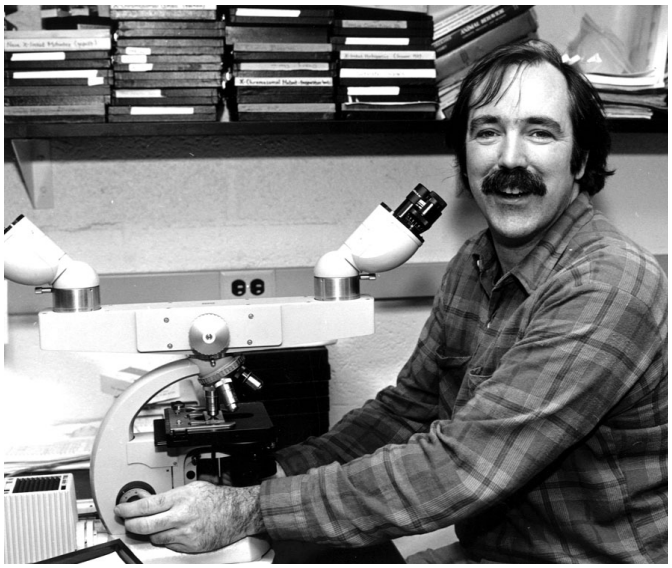
Overview

In this module you will accomplish great feats on a molecular level by employing Drosophila genetics. You will use a lethal transgene to create a population of pure virgin females (note: this is not for the faint-of-heart, as it involves genetically inducing the death of all their brothers). You will make a recombinant chromosome that makes flies permanently glow green. You will induce a transgene embedded in the fly genome to randomly jump to new places in the fly genome and you will identify the transgenes that have landed in desirable locations by using a Green Fluorescence Protein (GFP) reporter. You will also get experience using RNA interference (RNAi) in vivo.

You will learn about 6 Nobel Prizes:

1. Discovery of the chromosomal basis of inheritance
2. Discovery that scientists can make heritable changes to the genetic code
3. Discovery of all the genes required to instruct development of the embryo
4. Discovery of innate immunity
5. Discovery of GFP
6. Discovery of RNAi



See YouTube Video by Spring 2015 486H Student Pat Hartnett for History of Drosophila Nobel Prizes
https://www.youtube.com/watch?v=vYAVcxXVfME

You will learn how to employ state-of-the-art genetic techniques

1. How to set up genetic crosses in flies.
2. How to kill undesirable animals by inducing programmed cell death (apoptosis) with a heat-shock inducible lethal transgene.
3. How the yeast Gal4-UAS heterologous gene expression system works in flies to express transgenes in specific cells at specific times
4. How to mobilize transgenes using transposable element genetics
5. How to make a recombinant chromosome
6. How RNAi works

You will participate in the Culture of Science

Science is the business of creating and sharing information. The job of a scientist is to discover or create something new and share it with the world. There are many ways Drosophila geneticists share information and new things: we present posters and give talks at scientific meetings, we publish papers in scientific journals, and we deposit novel flies stocks to the national stock center in Bloomington Indiana. In addition, flies are routinely shared between labs, being sent either in the regular mail or by FedEx.

Every fly geneticist specializes in a specific area of biology. Some examples are: how embryos develop, how specific organs form, how cell shape and size is regulated, how organismal size is regulated, how flies fly without bumping into each other, how flies sense smells, tastes and temperature, how flies respond to toxins in the environment, how cancer develops, how aging is controlled. We tend to go to two types of meetings: (1) meetings based on topic, such as aging, which bring together researchers working on a variety of model organisms and (2) meetings for fly people, which brings together the entire community, which typically includes several people working on subjects similar to your own.

The major meeting for fly people is run by the Genetics Society of America and will be held this summer in Orlando Florida.

Fly Module Class 1. February 23, 2016

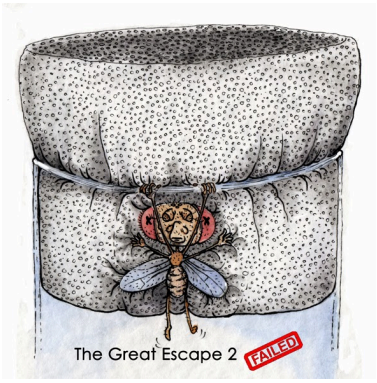
The Art of Fly Pushing

Today we will discuss Drosophila's place in the pantheon of model organisms. How it came to join the ranks of model organisms (it was first recognized as good for undergraduates) and what it's famous for (it has won 4 Nobel prizes so far), and why people keep using it (good for studying complex things like behavior and disease).

You will learn the basics of doing genetics with flies: How to tell the difference between females and males, how to set-up genetic crosses, and why it's essential to use virgin females.

You will learn about a genetic trick to get virgin females, using a Y-linked lethal transgene called heat shock Hid.

IN THE LAB



Part 1. Learn how to flip flies from one vial to another.

You will get two vials: one with **isolated females** and one with **isolated males**. You will get to experience putting them together, learning the art of tapping and flipping fly vials.

You will each get two vials:

1. **"isolated females"**
2.
3. **"isolated males"**

BEFORE YOU DO ANYTHING, BE SURE TO HAVE TAPE AND A SHARPIE TO LABEL YOUR VIALS!

For this exercise, practice tapping vials down so that the flies all gather at the bottom of the tube. As long as you don't break the vial, you don't have to worry about tapping too hard. The flies will survive!

After looking at the flies through the vial and seeing that they can survive taps, tap them down again and stick the vials in the ice bucket. After 1-5 minutes remove the vials from the ice. The flies should be sleeping now. If they are not, put them back in the ice bucket a little longer!

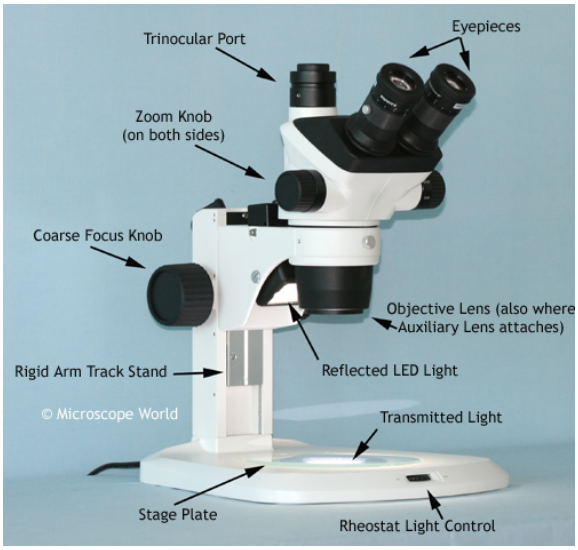
Once the flies are asleep, take the vials out of the ice bucket and put them on your bench top. Quickly remove the cotton tops and tap the female vial of flies into the male vial of flies. Put the cotton top back on the vial with all the flies right away! Afterwards, also put the cotton on the empty vial that had the isolated females. Keep both vials. The flies should wake up within 5 minutes.

Label each of the vials with your initials and the date.
One vial should be labeled “This Vial Contains females added to males”. The other vial should be labeled “This vial had Isolated females that were removed”

What do you predict these vials will look like in 2-3 weeks?
Be prepared to answer this before leaving class.

Part 2. Master the basics of fly laboratory equipment:

a. Dissecting microscope. First practice your ability to focus and zoom. The best sample to start with is a piece of paper with your name written on it. Check out the zoom and also try adjusting the lighting.



b. The CO2 pad and needle. You need to master this to be able to work with flies. You’ll notice there is a main tube from the CO2 tank running down the bench. From this main tube, there’s a T-connector bringing a tube to your microscope station. The red-handled valve controls the flow of CO2 from the main line to your microscope station. When the handle is perpendicular to the valve, it is shut. To open the valve, turn the handle so that it aligns with the valve. The tube after the valve splits by a Y-connector into two smaller tubes: one goes to the Fly Pad and one to the needle. Flow to the needle can be blocked by synching down the white clamp.

To get CO2 to your station, turn the RED handle so that it aligns with the valve. Make sure you can feel CO2 flowing from the needle. If you don’t, be sure the white clamp is open! Once you can feel a gentle flow of CO2 from the needle, you’re good to go!

See YouTube Video by Students from 486H Spring 2014
To See Firsthand How to Work with Flies and CO2
https://www.youtube.com/watch?v=HQ5ga4P40hc

Part 3. Gently put flies to sleep on onto your pad.

You will be given a vial of flies labeled "Practice Flies #1". Start with these!

Once you feel the flow of CO2 from the needle, you can put flies to sleep and have them continue sleeping on the fly pad. There are two things you need to do: (a) first get flies to sleep in the vial or bottle and (b) tap the sleeping flies onto the fly pad, where they will continue sleeping.

a. Getting the flies to sleep in the vial or bottle

*****REMEMBER THIS: Hold the vial or bottle of flies UPSIDE DOWN the whole time, until you tap flies onto the pad.** ***

Pick-up the needle with CO2 flowing out, and slip it between the cotton and side of the vial, or bottle top and side of the bottle. Keep holding the vial/bottle UPSIDE DOWN. Gently tap the sides of the vial/bottle. Wait for all the flies to fall onto the cotton/bottle top. Then wait a few more seconds (e.g. count to 10). Then remove the needle and gently tap the flies onto the fly pad. Once the flies are on the pad, close the white clamp so that all the CO2 goes to the fly pad.

b. Putting flies on the pad.

Remember this: *You do not ever have to put all the flies on the fly pad. Put no more than the number that would fit inside a circle the size of a quarter (maybe 50 flies max).

Once you tap sleeping flies onto the fly pad, be sure to put the cotton back in the vial/or plug in the bottle and keep the vial/bottle on its side. The reason for this is that some flies may still be inside, and putting the bottle upright could kill them because they could fall onto the food and get stuck there forever.

c. Using the paintbrush. Use the paintbrush to move flies around on the fly pad. Try gently turning one fly over to see its stomach and then its back. This is not painting, so do not hold the paintbrush like you are painting. Hold the paintbrush more like a pen, close to the business end, the bristles. You'll do best if you rest your hand on the table as you use the brush. This will steady your hand so that you can gently push and roll the flies without killing or damaging them.

d. Get to know the flies.

Zoom in to see their eyes as closely as possible. Look at the pattern of bristles on the fly's back. See if you can find the triangle shaped cuticle on the fly's back – if so, how many bristles do you see on it? What segment or segments do each pair of legs originate from? Can you tell the difference between females and males? Don't be afraid to look closely!

e. Return flies to their vials

When you feel that you have a good feeling about how flies look up close, try returning the sleeping flies to their vial. Brush them gently to the edge of the fly pad. Then open the vial that you got them from and gentle tap them into the vial. Put the cotton back in and rest the vial on its side.

f. Learn to flip flies to a new vial. To do this, get a new vial!
Once the flies are fully awake, try flipping them to a new vial using the tapping method without using CO2 or cold. Get the new vial and ask the TA or Prof to show you how. Then try it yourself, flipping flies back and forth until you are comfortable flipping flies.

g. Label your vial of Practice Flies #1 with your initials and the date.
On Thursday, see how many flies are still alive. This will give you an idea of how much of a gentle giant you naturally are with flies. If you're not 100% gentle, don't worry, you can (and will) improve!

FAQs: read this before you continue with the lab exercises!

What if flies start to wake up?

There's different levels of flies waking up, so look at this list carefully and be prepare to take appropriate action!

If flies are staring to twitch!
If flies start twitching on your pad, make sure the white clamp is synched down. Also, cup your hands over the flies to concentrate the CO2 around them.

If flies are starting to walk!
If flies start to walk, you're in trouble. As fast as possible, brush them into a vial! If they are walking off the pad, you must kill them. See Massive Wake Up Catastrophe!

Massive Wake Up Catastrophe
If flies are starting to stream off your fly pad, there's no other solution than to kill them all. Ask for help. But if no one's around, you'll have to do it yourself. Your choices are to crush them with your hand (with or without paper towels) or to drown them with a handy squirt bottle of water or ethanol. If you use the drowning method, you will have to follow-up with paper towels to clean them off the bench top. If you use the crushing method you will have to follow up with ethanol, to clean up the mess. And, please wash your hands after!

Part 4. Learn how to sex flies

The first job of any fly geneticist is to be able to tell the difference between females and males.

You will be given a vial of flies labeled "Practice Flies #2". Put these flies to sleep and onto the fly pad!

Use your brush to push all the flies to the center of the pad. Then gently push one fly apart from the pile so that you can look at it closely. Use the brush to turn the fly on its back and then look closely at the genitals to see if it is female or male. Once you figure out the sex of this fly, get another fly from the pile that is of the opposite sex. Put the flies side by side on their backs and be sure they are different sexes. Ask a TA or Prof to double-check that you got it right.

Once you get the OK from the TA or Prof, sort the rest of the flies into three distinct piles:

1. "Females"
2. "Males"
3. "not sure"

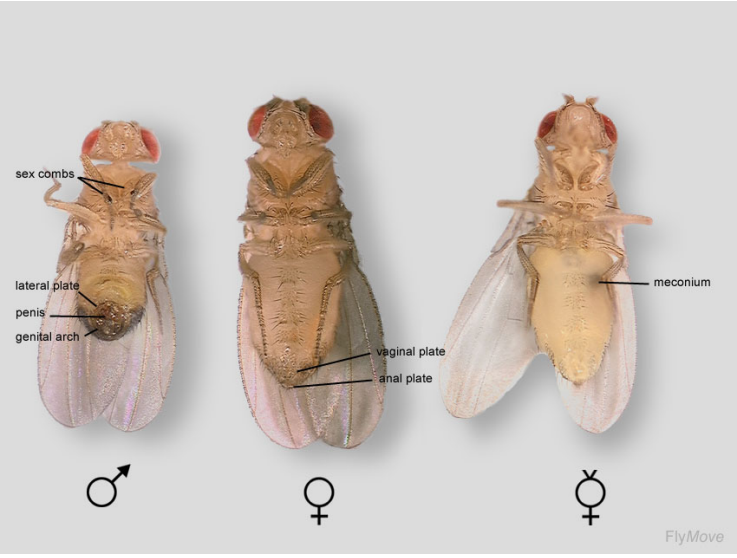
Ask a TA or Prof for help with any flies in the "not sure pile"

Next, have your bay mate double-check your work. Once they give you the OK, get a TA or Prof to check your work.

You must get this 100% correct to continue with the class!

Put flies in new vials and label with your initials and the date – as follows:
After you get the OK from a TA or Prof, gently push all the females into a new vial labeled with "Your Initials, Sorted Females, Date." Then gently push all the males into a different new vial labeled with "Your Initials, Sorted Males, Date". Keep the vials on their sides until the flies wake up.

What do you predict will happen in each of these vials over the next 2-3 weeks?



Part 5. Killing males with the power of genetics.

You will receive a vial with **hs-Hid flies**. There will be a mixture of females and males in each vial, with about 15 adult flies in total. These adults are the parents for your experiment and they are continuously mating and laying eggs. The vial therefore has not only the adults, but the embryos they have been laying over the last couple of days, since being put into the vial. Some of those embryos will have hatched, giving rise to small larvae. *And if you haven't figured out the answer to the questions in red above, this is hopefully a good hint!*

You will learn how to selectively kill males using the Y-linked hs-hid transgene.
To do this you have to get a collection of 0-20 hour old embryos. The trick is to let flies lay eggs in a vial for 0-20 hours and then to remove the adult flies. What you'll be left with is a collection of their embryos. You'll have to figure out how to do this so that when you come to the next class at on Thursday at 1 PM, you have your very own collection of embryos that are anywhere from 0-20 hours old.

For ideas see Student Lab Reports from 486H Spring 2015 in the Appendix.

Hint: You have to come in for 5 minutes on Wed.

The next class starts at 1 PM on Thursday. If you put flies in a fresh vial on Wed at 1 PM, then by 1PM on Thursday, the embryos they have laid will be anywhere from 0 hrs old (if laid as you were walking in the door on Thursday) or 24 hours old (if laid when you put them in the vial on Wed at 1PM). But you want the collection to not be more than 20 hours old. So, what is the LATEST time would you want to flip flies into a new vial on Wed? _____PM.

On Wed, you will flip your hsHID flies to a new vial. Label the new vial with tape that has the name of the fly stock, your initials, the date, AND THE TIME OF THE FLIP. Put both the new and old vial back in the 25 degree incubator.

Before you leave, you must figure out whether and when you can come in tomorrow, on Wed. Feb 24, to flip your flies. This will take less than 5 minutes of your time. Discuss with a TA or Prof before leaving!

Before you leave make sure all your vials are labeled, organized, and stored in the 25°C incubator.

You should have:

1. *Vial of males with females added*
2. *Vial that had isolated females (no adults left)*
3. *Practice Vial #1 (all adults still present)*
4. *Practice Vial #2 (no adults left)*
5. *Sorted Females*
6. *Sorted Males*
7. *hsHid stock of flies*

Fly Module Class 2. February 25, 2016

Getting Virgins the Easy Way & Setting up a Gal-UAS Genetic Cross

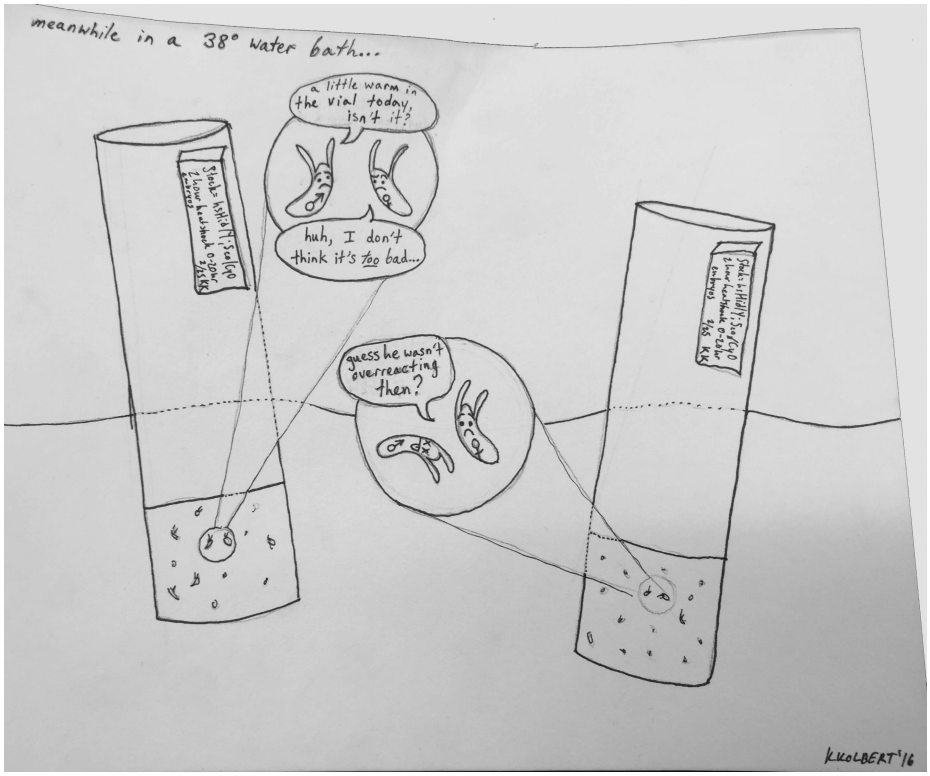
Today we will discuss transgenes, how genes are transcribed, and the Gal4-UAS system. This system enables Drosophila researchers to express any transgene in any tissue at anytime. My lab uses this system to create models of colorectal cancer in the fly intestine. We use a stem cell expressed Gal4 transcriptional activator, or "driver" to turn on the expression of three transgenes: UAS-GFP, UAS-Raf-oncogene (induces cancer), and UAS-Luciferase. This is a powerful way to create tumors that we can track by both GFP expression and luciferase activity. If time permits we will also discuss the Chi-Squared Test, which may be a useful tool for you in future labs!

You will set up two experiments:

(1) the heat-shock experiment to get virgin females and

(2) A genetic cross between Gal4-driver flies and UAS-GFP responder flies. This cross is actually step one of making a recombinant chromosome, which we will discuss right before spring break.

In lab you will have an opportunity to look at prepared slides on the upright fluorescence microscopes, to see stem cells uniquely marked using the Gal4-UAS system.



See You Tube video by 486H students
"My Heat Shock Kills All The Boys In The Vial"
https://www.youtube.com/watch?v=cEYLak29iO8

IN THE LAB

Part 1. Perform a 2 hour heat-shock at 38°C

The easiest way to get virgin females is to kill their brothers before they are born. An elegant way to do this is with genetics that takes advantage of a lethal gene on the Y-chromosome. Since only males have a Y-chromosome, this is a way to kill all the males effortlessly. Of course, if we kill all the males we'd be out of business as geneticists, so we want to be able to kill the males selectively. The lethal gene we will use is INDUCIBLE. It is normally not expressed, but if we turn up the temperature it gets turned on. Why? Because it is under the control of a "heat-shock promoter" – it is turned on by transcription factors (called heat-shock factors) that become activated at temperatures over 32°C.

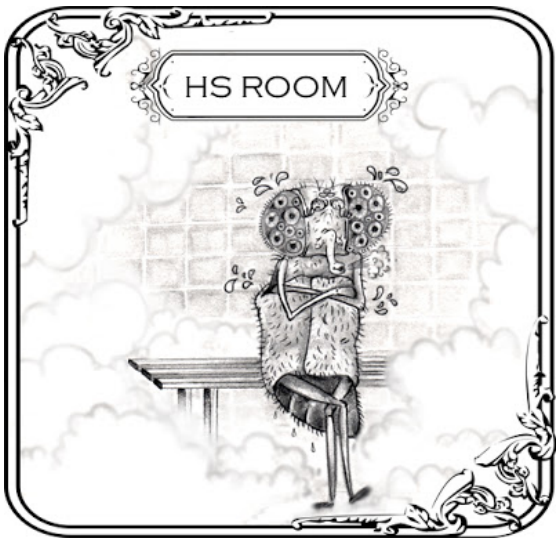
The flies we are working with have the following genotype:

Females: w/w;+;+
Males: w/Y-hsHID;+;+

How to perform the heat-shock:
Before putting the vial in the water bath, flip the adults to a new vial! Label the vial new with a piece of tape that has your initials and the date and time and the genotype of the flies. Take the "empty" vial, with the collected embryos and submerge in the water bath for 2 hours. Do not drown the flies! Keep the tops of the vials above water!

You will then take the heat-shocked vial and keep it at 25°C. Label the new vial with your initials and the date of the heat-shock.

Optional control for your experiment:
The vial with hsHid adults should be flipped on Friday before 1 PM. This can serve as your No Heat Shock Control, should you decide to have such a control. You can also do this next week….



What to expect:
If the hsHid stock is 100% Effective, you will expect the heat-shocked embryos to give rise only to females. There should be zero males. However, your stock may not be perfect. There may be 1% males, or 5% males, or some other percentage of males.

Any males that you find after heat shock are called "escapers" because they escaped their genetic fate. (For those of you taking Biol 283: This is because the lethal transgene is not 100% penetrant.) However, not to worry – escapers will very likely be sterile!

What to do after the heat-shock?
Look at the vial at the start of class next Tuesday March 1 and Thursday March 3 to see how flies grow and develop. Adult flies will start to hatch about 10 days after you started your experiment, which will be March 7. We expect that all will have hatched by March 10. To determine how effective your stock is at killing males, count the number of progeny and record how many were female and how many were male.

If you did the control, count how many females and males hatched from the control vial.

Doing good science:
We claim the females you will get from the hsHid experiment will be virgin. We further claim that any male escapers that might arise after heatshock will be infertile.
How can you test either of these claims?

Planning Ahead: Draw or include a calendar.

Mark when embryos were first heat-shocked. Then show on the calendar when you expect them to hatch (10 days later). Then mark when you expect them to all be hatched (14 days from the start of the experiment). Then indicate that this is when you will count the progeny.

Before you leave

1. You should have two vials from the heat shock experiment, labeled as follows.

Make sure you label your heat-shock hid heat-shocked vial with the GENOTYPE of the flies, your initials and the date. Include the time of the egg collection in hours. For example: "**w/hsHid/Y. 2 hour heat-shock of 0-20 hour embryos 2/25. MM**"

Label the other vial with the hs-hid adults, with the genotype, your initials and the date. Write: No Heat-shock.

Put these vials at 25 degrees with your other vials.

2. You should plan on coming in tomorrow, briefly to flip the adult hs-Hid adults to a new vial. This way you will have a control collection of fly embryos that are about 0-20 hours old and NOT heat-shocked. WRITE DOWN THE TIME YOU PLAN TO COME IN TOMORROW TO DO THE FLY FLIP.

3. WRITE DOWN WHEN YOU EXPECT TO GET THE RESULTS OF YOUR EXPERIMENT. Answer: March 10th. However, look at the vials every class to track development of the flies and to add water to the vials if necessary to help the larvae develop. This may be necessary because in the winter the food is sometimes dry and needs water added so that the larvae can chew it up and survive.

Your Notebook for the Heat-shock Hid Experiment should include the following:

1. Goal
Write in normal sentences what your goal is. For example: My goal is to test how efficient the w/Y-hs-Hid stock is at killing males.

2. Expectation
I expect that the stock will be perfect at killing males when the killing protocol is followed correctly. I expect that this stock will not kill any males when the killing protocol is not implemented.

3. Experimental Approach
To determine how well the stock kills males, I will follow the heat-shock protocol as follows:

Obtain a collection of embryos that are 0-20 hours old. I did this as follows: Explain what you did

Heat-shock the collection of eggs for 2 hours at 38°. I did this as follows: Explain what you did

Control: I also tested a 0-__? hour collection of eggs that had not been heat-shocked. I did this as follows: Explain what you did

I scored the results by counting the number of female and male offspring that hatched as flies after heat-shock vs. no heat-shock.

4. Results. You will get these before Spring Break.
Write a chart showing the number of females and males obtained from each population of eggs you collected.

Also enter your data on a collective chart for the class.

5. Analysis. Write your interpretation for your own dataset. Then write an interpretation for the complete dataset for your stock, using the collective data of the class.

Optional: Try your hand at applying a Chi-squared analysis. Are your results statistically significant?

IN THE LAB

Part 2. Plan out your crosses!
Gal4-Driver Flies to UAS-GFP Responder Flies

A. Get three vials of flies:

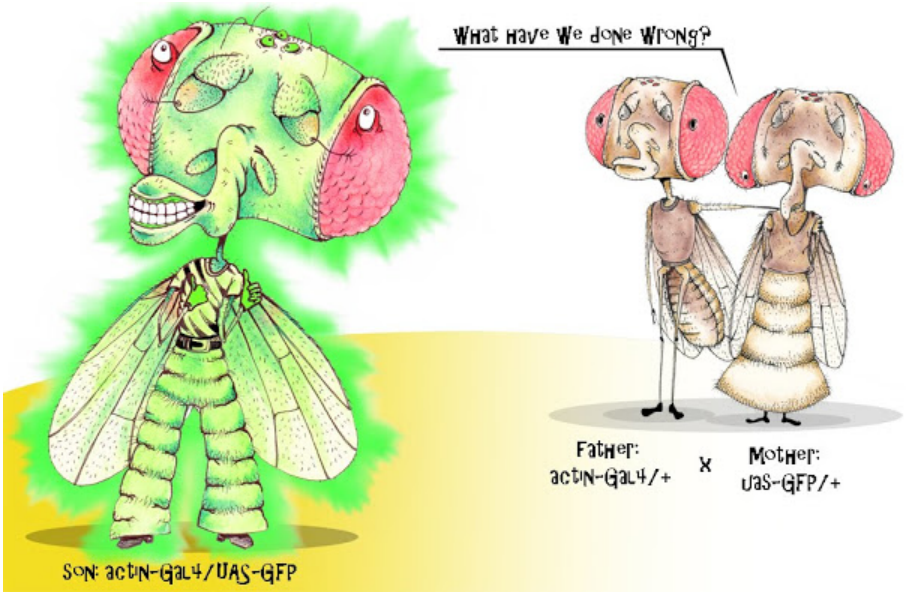
1 vial of virgin females with UAS-GFP on chromosome 2: w/w;UAS-GFP/UAS-GFP;+/+

1 vial of virgin females with UAS-GFP on chromosome 3: w/w;+/+;UAS-GFP/UAS-GFP

1 vial of Gal4 Driver males with the driver on chromosome_____ (fill in blank)

B. Write the genetics of your cross of the Gal4 driver line to the UAS-GFP responder on Chromosome 2. Show the genotypes of the parents and the expected F1 progeny. **Do you expect any of the F1 progeny to glow green?**

C. Write the genetics of your cross of the Gal4 driver line to the UAS-GFP responder on Chromosome 3. Show the genotypes of the parents and the expected F1 progeny. **Do you expect any of the F1 progeny to glow green?**



BEFORE YOU CONTINUE, HAVE A TA OR PROF CHECK YOUR WRITTEN GENETICS!

_____

initials of person who checked your work

Part 3. Set up your crosses!
Gal4-Driver Flies to UAS-GFP Responder Flies

Step 1: Get two empty vials of fly food.
Get tape and write out each cross, plus your initials plus the date.

Your labels can be written as:
INSITE GAL4#__Males X w;UAS-GFP;+ Females 2/25, MM (your initials)

INSITE GAL4#__Males X w;+;UAS-GFP Females 2/25, MM (your initials)

Step 2. Distribute your Gal4 driver males into two new vials.
Use CO_2 to put the males asleep in the vial upside down. Tap the males out of the vial and on the pad and put into two piles of 4+ males in each pile. Then use the brush to scoop them into new vials. Keep the vials with sleeping males on their side.

Step 3: Distribute your Chromosome 2 UAS-GFP Responder Females to the appropriate vial of males.
Use CO_2 to put the females asleep in the vial upside down. Tap the females out of the vial and on the pad and put into a pile of 7-10 females. Then add a little pulse of CO_2 to the vial of males with the lable for the chromosome 2 cross. Then use the brush to scoop the females into this vial of sleeping males. Keep the vial of sleeping males and females on its side.

Step 4: Distribute your Chromosome 3 UAS-GFP Responder Females to the appropriate vial of males.
Use CO_2 to put the females asleep in the vial upside down. Tap the females out of the vial and on the pad and put into a pile of 7-10 females. Then add a little pulse of CO_2 to the vial of males with the lable for the chromosome 3 cross. Then use the brush to scoop the females into this vial of sleeping males. Keep the vial of sleeping males and females on its side.

Plan Ahead Draw or include a calendar. Mark when you set up the crosses. Then show on the calendar when you expect to be able to see the L3 (5 days later). Show on the calendar when you expect to have adults (10-14 days later).

Note: This experiment is not only to see the power of Gal4, but it is also to make a **RECOMBINANT CHROMOSOME**. You will therefore need to COLLECT VIRGIN F1 FEMALE PROGENY FROM THIS CROSS. And since we don't have a lethal Y chromosome, you have to do it the old fashioned way, which is using the clock method to get females before they are old enough to cross with their brothers. **So, put STARS in your calendar, indicating when you will start to collect virgins…10 days after today!**

Thinking ahead:

What % of the F1 offspring do you expect to fluoresce green?_____

If the F1 progeny develop according to plan, they will be L3 larva on Tuesday March 5th. You will be able to inspect these flies to see what % are green!

Thinking ahead, after you get the F1 progeny adult flies (starting March 7 or 8): If you cross a Green Fluorescing F1 progeny to a fly that does not have any Gal4 drivers or UAS-GFP, what percent of the F2 progeny from that cross do you expect to fluoresce green?

Before you leave

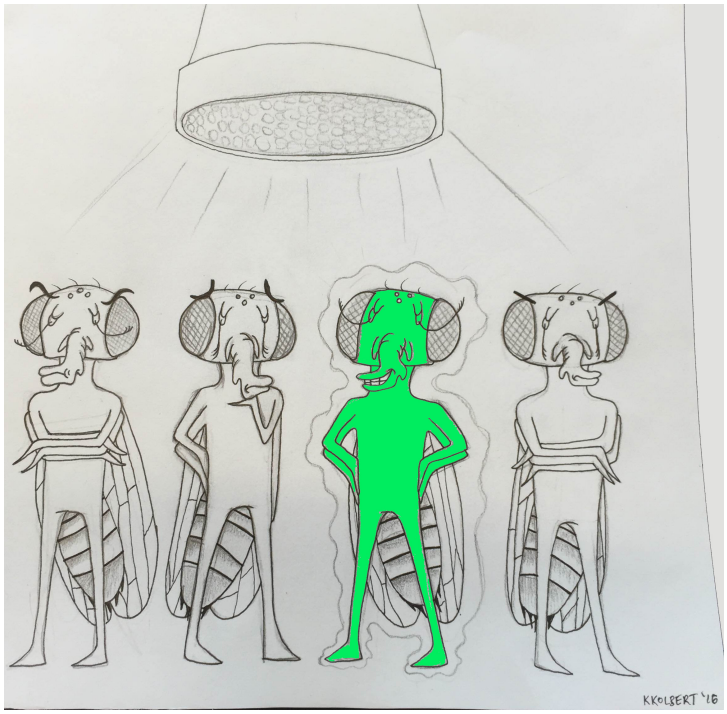
Before *you leave make sure all your vials are labeled, organized, and stored in the 25°C incubator.*

Label the vial of the crosses with the genotypes of the females and males, your initials and the date. Keep the vials at 25 degrees. Flip the adults to a new vial on Sunday or Monday and keep both vials at 25 degrees.

Fly Module, Class 3. March 1, 2016

The Power of GFP and How To Make Transgenic Flies

In today's class, we will first discuss why the discovery of Green Fluorescent Protein (GFP) was awarded the Nobel Prize. We will set up the GFP blue light flashlights and analyze the results of your Gal4 X UAS-GFP crosses that you set up on Thursday February 25th. You should look at your data to see the pattern(s) of GFP that are visible in the L3 larvae, and to determine if some or all of the larve exhibit the same GFP pattern. Discuss your results with a TA and/or Michele.



After looking at your GFP results:
We will then discuss new material concerning how transgenics are made in Drosophila. There are over 100,000 different transgenes (encoding unique DNAs that were made in the lab) that have been engineered into the Drosophila genome. All engineered transgenes are first introduced into the Drosophila genome by injection of DNA into fly embryos. However, once a transgene is introduced into the fly genome by injection, it can later be mobilized to new areas of the fly genome by crossing the transgenic fly to a fly that expresses a special enzyme, called a Transposase. Transposases can: (1) recognize transgenes, (2) cut transgenes out of the genome, and (3) splice transgenes into new locations in the genome. Transposase is especially useful for special genetic screens called Enhancer Trap Screens. We will discuss the genetics and molecular biology of enhancer trap screens in preparation for class on Thursday, when you will each set up an enhancer trap screen to create and identify transgenes that express the Gal4 repressor, called Gal80 in specific tissues.

Fly Module, Class 3. March 1, 2016

IN THE LAB

Part1:
Document the results of your Gal4 X UAS-GFP crosses. What percent of flies glow green in each cross?

Part 2:
Check on the progress of all your flies. Ask us any questions you have!

Part 3:
Discuss and submit a well-thought out draft of your Fly Module Project

Lastly, each of you will have to do a Fly Module Project either individually or within a group of up to 4 students. We will brainstorm together and by the end of the class period you will submit a draft proposal for your project. Your Fly Module Project will be presented in class on the last day, March 28.

Before leaving make sure to have Michele sign-off on your plan!

_____

M. Markstein initials

As always, before you leave…

Before you leave make sure you have inspected all your vials and have added water if necessary. Be sure to put them all back in the 25°C incubator.

Fly Module, Class 3. March 1, 2016
Fly Module Project Ideas

You have the opportunity to create something that will be used by the next generation of students in the class and/or the larger fly community!

Here are some ideas of what you can create:

1. A Poster or Video on the hs-hid experiment

2. A Poster or Video on how the Gal4-UAS system works in Drosophila

3. A Poster or Video on how to make Gal4-GFP recombinant chromosomes

4. A Poster or Video on Drosophila names. e.g. brain mutants: turnip, cabbage, blue-cheese, swiss-cheese), sonic hedgehog and the threats of legal action by SEGA.

5. A Poster or Video on Drosophila and American Politics (Two major Republican politicians have trashed “fruit fly research”. This can also discuss how science is funded.

6. A Poster or Video on Drosophila and the Nobel Prizes it has won

7. A Poster or Video on the Drosophila Gal80 Enhancer Trap Screen

8. A Poster or Video on using Drosophila for transgenic RNAi experiments (you will have to read ahead to propose this today).

9. Edit Wikipedia Entries on Genetics: Improve the page for Calvin Bridges. Improve page for Genetic Epistasis.

10. Write an Instructional “How To” regarding some aspect of this laboratory for future students.

11. Propose other ideas!

Before you leave submit your proposal to Michele

What is the goal? Who is doing what exactly? What is your timeline for different aspects of the project? Who is the intended audience?

Fly Module Class 4. March 3, 2016

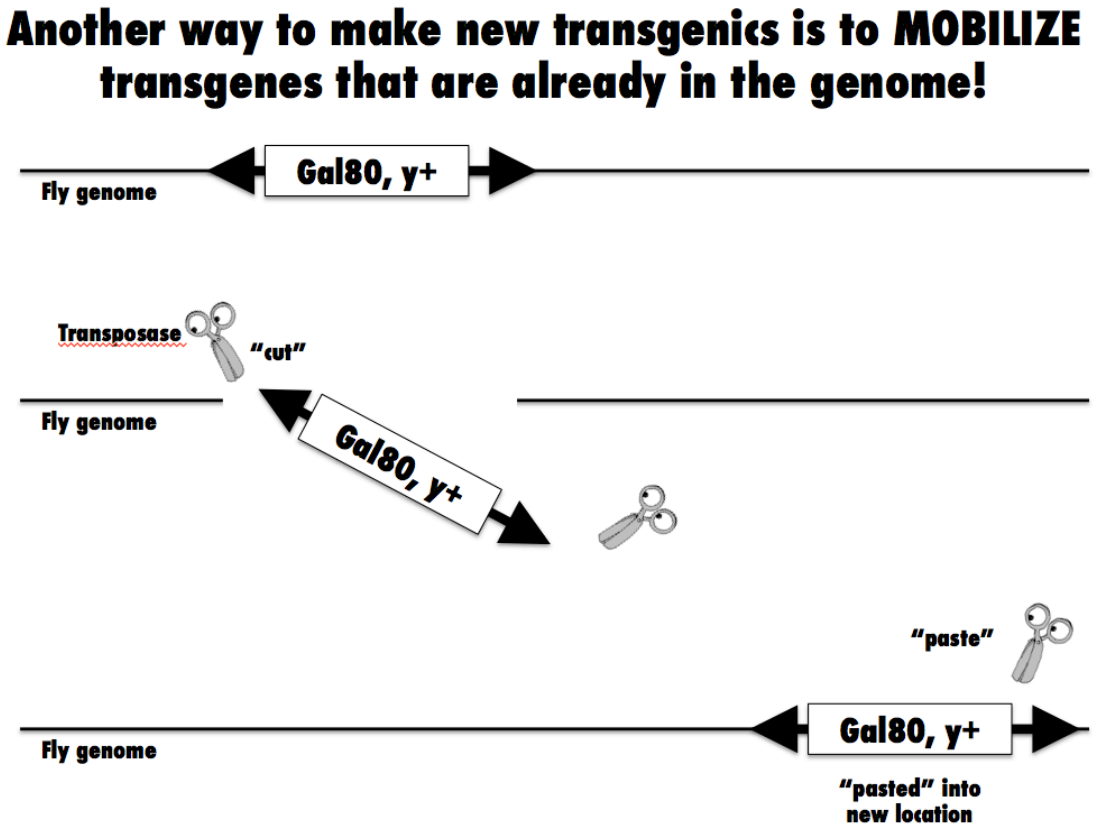
A Genetic Screen for Gal80 Enhancer Traps

In the previous class we will have discussed how P-elements are mobilized with the delta 2-3 transposase. You should come to class prepared with an understanding of how that works. In today's lecture we will discuss using the delta 2-3 transposase to mobilize a specific transgene that encodes the Gal4 repressor called Gal80.

One of the great strengths of the Gal4 system is that it enables us to express transgenes in specific tissues. However, almost every Gal4 driver is active not only in a specific tissue or cell type, but also in the salivary glands of both the larvae and adults. The reason for this is that the minimal promoter that was engineered into the Gal4 transgene has a "cryptic" salivary gland enhancer. To correct for this problem, we would like to create Gal80 enhancer trap flies that express Gal80 in the salivary glands. In lecture we will discuss how to do an enhancer trap screen.

In the lab portion of the class, you will set up the crosses for the screen and when you come to the next class on Tuesday March 8, you will screen the L3 Larvae for Gal80 enhancer traps.

This work, if successful, will lead to a publication in the scientific literature.



IN THE LAB

Part 1. Plan out your Crosses!

P{Gal80,y+}/Delta 2-3 males
X
UAS-GFP;SG-Gal4 females

A. Get 6 vials of flies:

1 vial of 20 males
yw/Y; (If or CyO)/+; P{Gal80,y+}/Delta 2-3,Sb

4 vials of 25 females each
y+w; UAS-GFP; SG-Gal4

1 vial of a control cross already set up for you:
male yw/Y; +; P{Gal80,y+} X female y+w; UAS-GFP; SG-Gal4
these males have no transposase.

B. Write the genetics of your control cross. Show the genotypes of the parents and the expected F1 progeny. **Do you expect all of the F1 progeny to glow green?**
C. Write the genetics of your screen crosses. Show the genotypes of the parents and the expected F1 progeny.
Do you expect all of the F1 progeny to glow green? If not, why not? What would the genotype of such an F1 look like? Draw a hypothetical chromosome showing an enhancer trap! What will you do if you find a fly that is not glowing green?

Part 2. Set up 20 Crosses for your Screen!

A. Get 20 empty vials of fly food.

B. Write 20 labels on tape using Michele's special factory-proven method. You will save a lot of time this way. Each label should read:

> **Gal80,y+/Delta2-3,Sb male X UAS-GFP;SG-Gal4 female**
> **Unique # (1-20), Your initials, 3/3**
>
> *NOTE: **Write these labels in duplicate** so that you can easily transfer the adults and the label to a new vial on Sunday or Monday. Put two identical labels on each vial.*

C. Add 1 male Gal80,y+/Delta2-3,Sb per vial.
Use CO2 to put the males to sleep in the vial upside down. Tap the males out of the vial and on the pad. Then use the brush to scoop one male into each new vial. Keep the vials with sleeping males on their side.

D. Add 5 female UAS-GFP;SG-Gal4 flies per vial.
Use CO2 to put the females asleep in the vial upside down. Tap the females out of one vial and on the pad and put into 5 piles of 5 females. Then add a little pulse of CO2 to 5 s vials of males. Then use the brush to scoop the females into each of the 5 vials of sleeping males. Keep the vials of sleeping males and females on their sides until the flies wake up. Repeat this with each vial of females until all 20 crosses are set-up.

E. Put your crosses in your box at 25 degrees.

F. Plan to flip these crosses on Sunday or Monday to new vials. You can easily peel off one of your new labels from the original vial to the new vial when you do the flip

PART 3. LOOK AT THE VIALS OF YOUR CROSS OF INSITE-Gal4 X UAS-GFP.

You will need to collect 3-10 virgin females to be able to make a recombinant chromosome! Review your vial with Michele to see when to expect virgins to hatch!

Plan Ahead Draw or include a calendar.

Mark when you set up the crosses. Then show on the calendar when you expect the be able to see the L3 (5 days later). Show on the calendar when you expect to have adults (10-14 days later).

Before you leave

Before *you leave make sure all your vials are labeled, organized, and stored in the 25°C. Have a plan of when to flip your crosses on Sunday! Have a plan of when to start collecting virgins!*

Your Notebook for the Gal80 Enhancer Trap Experiment should include the following:

1. Goal
Write in normal sentences what your goal is. For example: My goal is to create new enhancer traps in which the Gal80 expressor is expressed in the salivary gland.

2. Experimental Approach
Explain how transposase works.
Explain how Gal80 works.
Write the genetics of your cross: the genotypes of the parents, and the expected genotypes of the offspring.

Explain why you are using a UAS-GFP;SG-Gal4 reporter
Explain the point of the control cross you were given

3. Results. You will get these before Spring Break.
Make a table for your results for the 20 crosses you set up. For each vial indicate whether their were progeny; indicate how many L3 you screened; indicate how many Hits you found.

4. Analysis.
What was your hit rate in terms of the number of crosses you set up? How about in terms of the number of L3 larvae that you screened?

5. Thinking ahead.
Do you think that every hit that shows Gal80 activity in larval salivary glands will also show Gal80 activity in adult salivary glands? Why or why not?

Fly Module Class 5. March 8, 2016

Conduct the Gal80 Enhancer Trap Screen and Set up an RNAi screen

In lecture we will discuss RNAi as a genetic tool to knockdown the expression of specific genes. The fly community is heavily invested in RNAi and has three genome-wide RNAi libraries: one made at Harvard, on in Japan, and one in Vienna.
Each has about 10,000-20,000 fly stocks lines, likely in duplicate or triplicate.

You will have a chance to use RNAi to test whether a particular gene is essential (meaning necessary for life) in Drosophila. In my lab we recently identified genes that are required in stem cells for multidrug resistance. Your job will be to test whether they are required more broadly for survival of the flies. In our discussion we will talk about the biology of RNAi and the multidrug resistance genes you will be investigating.

IN THE LAB

Part 1. Collect female virgin flies from your cross of INSITE-Gal4 X UAS-GFP.

Specifically you will want to get flies that have INSITE-Gal4 and UAS-GFP on HOMOLOGOUS CHROMOSOMES.

Part 2. Screen for enhancer traps that block the activity of Gal4 in the salivary glands.

P{Gal80,y+}/Delta 2-3 males
X
UAS-GFP;SG-Gal4 females

A. First look at your control cross:
male yw/Y; +; P{Gal80,y+} X female y+w; UAS-GFP; SG-Gal4

All of the L3 larval offspring should have Green Glowing Salivary Glands.

B. Screen your 20 screen crosses of:
Gal80,y+/Delta2-3,Sb male X UAS-GFP;SG-Gal4 female

If you find any L3 that do not have glowing salivary glands, circle the larvae using a Sharpie and call Michele over right away! She will then retrieve the larvae into a new vial. Any larvae that fail to express GFP are considered “hits” in your screen.

C. Count the number of L3 larvae that you screened in each vial so at the end you can say how many you screened. You may find it useful to mark each L3 with a dot, or circle each one, as you count all the L3 in a vial. This is good to do because it tells us how likely someone is to get hits in the future with this set up and also, it will give you bragging rights, if you ended up screening hundreds of L3!

D. You now have enough data to write up your lab report!

Part 3. Set up your Gal4 X UAS-RNAi cross

A. Get two vials of flies:

Vial 1: Males: yv;+;UAS-RNAi (against a specific transporter)
Vial 2: Virgin Females: Actin-Gal4/CyO

B. Write the genetics of your cross including the genotype of the expected offspring. Write your expectations if the transporter is either essential (required for like) or inessential (not required for life).

C. Get an empty vial with fly food and write duplicate labels on tape with the cross, your initials, and the date. So, put two labels on the vial!

D. Set up the cross using about 5-10 females X 5-10 males

Plan Ahead Draw or include a calendar.

Mark when you set up the crosses. Then show on the calendar when you expect the be able to see the adults. 10-14 days later.

Before you leave

Before *you leave make sure you have talked to Michele about your hits from the Enhancer trap screen.*

Return your vials to 25°C.
Have a plan of when to collect virgins of Insite-Gal4/UAS-GFP if you still don't have enough!

Your Notebook for the RNAi Experiment should include the following:

1. Goal
Write in normal sentences what your goal is. For example: My goal is to determine if the ABC transporter _____ is essential for fly survival

2. Experimental Approach
Explain how RNAi works.
Explain what ABC transporters are.
Draw the genetics of your cross: the genotypes of the parents and the genotypes of the expected offspring.

3. Results. You will get these after Spring Break!
Record the number of progeny that have Cyo wings and straight wings.

4. Analysis.
What do your results tell you regarding whether the transporter you are studying is essential or not?

5. Thinking ahead.
Why would a gene not be essential to an animal? Would you expect most genes to be essential or not?

Fly Module Class 6. March 10, 2016

Make a recombinant chromosome!

You will use fly genetics to create a recombinant chromosome that has both Gal4 and UAS-GFP together on the same chromosome.

How can you make a recombinant chromosome with only a paintbrush? Well, you let the fly do it for you and then you take all the credit!

You are already familiar with the biology of making a recombinant chromosome – it's simply the process of recombination that occurs during Meiosis. Remember how the homologous chromosomes line up and form tetrads? And how there's crossing-over between the maternal and paternal homologs within the tetrad? Well that's what we're going to rely on – recombination during meiosis between the chromosome with the Gal4 driver and the chromosome with the UAS-GFP.

The specifics of the recombinant chromosomes you will be making:
This is not merely an exercise: the Gal4 driver lines you will be working with are novel and soon-to-be-published. They were created by 486H students in 2012. These flies are now at Lawrence Berkeley Labs being sequenced so that we can know the exact location where each of the Gal4 transgenes has integrated into the genome.

IN THE LAB

Part 1. Get a vial with male yw;L/Cyo;Msl3/TM3,Sb

Part 2. Write out the genetics of your cross

You will have collected the virgin females of the following genotype:
INSITE-Gal4#___/UAS-GFP.

You will cross these to males that are yw;L/Cyo;Msl3/TM3,Sb

If there is no recombination, what do you expect the progeny to look like in terms of GFP expression?

IF there is recombination, what do you expect the progeny to look like in terms of GFP expression?

Are there any recombinant chromosomes that would not result in GFP glowing flies?

BEFORE YOU SET UP YOUR CROSSES,
HAVE A TA OR MICHELE CHECK YOUR WORK.

Part 3. Set up your crosses:

Depending on how many virgin females you have, you will set up 1-3 crosses, with each cross having 1-3 females.

A. Get the appropriate number of empty vials with food.

B. Write out your labels on tape, in duplicate, with the cross, a # to identifiy the cross, your initials and the date. Write the labels in duplicate so we can flip your cross for you over the Spring Break.

C. Add the females to the vials.

D. Add an equal number of the males to the vials. Keep the vials on their sides until all the flies wake up and then store at 25 degrees.

Plan Ahead Draw or include a calendar.

Mark when you set up the crosses. Then show on the calendar when you expect the be able to see the adults and the L3. We will flip the crosses into new vials so that you can see the L3 when you return from Spring Break!

Part 4. Get results from your w/Y-hsHID experiment. Count the number of female and male progeny.

You are now ready to finish that lab report!

Part 5. Flip your RNAi cross (or flip it on Friday).

Part 6. Work on your Module Projects.

Before you leave for Spring Break:

Make sure your crosses to make recombinant chromosomes are clearly labeled with DUPLICATE LABELS. Add a "FLAG" to those vials so we can easily locate them and flip them over the break. Ask Michele or TA to check over your vials before you leave.

ALSO: Give Michele a progress report on your MODULE PROJECT

Your Notebook for the recombinant chromosome experiment should include the following:

1. Goal
Write in normal sentences what your goal is. For example: My goal is to make a recombinant chromosome with Insite Gal4 ____ and UAS-GFP.

2. Experimental Approach
Draw the genetics of your crosses: The first cross which you performed on Feb 25 and the second one which you performed March 10.

3. Results. You will get these after spring break!

4. Analysis.
What do your results tell you regarding whether the transporter you are studying is essential or not?

5. Thinking ahead.
Why would a gene not be essential to an animal? Would you expect most genes to be essential or not?

Fly Module Class 7. March 22, 2016

Recover recombinant chromosomes!

No planned lecture.

IN THE LAB

PART 1. Screen the L3 and adults for recombinant chromosomes.

Use the Blue Light GFP flashlight to screen the L3 and adults for GFP fluorescence. IF you get any hits, let Michele know! Michele will likely ask you to retrieve them into a new vial.

Count the number of hits and the L3 screened so that you can calculate your hit rate.

You now have enough data to write you're your Recombinant Chromosome Report.

PART 2. Check your RNAi vials.

If all progeny have hatched, count the number of Curly winged and straight-winged progeny to figure out whether or not the transporter you are tested is essential for life or not. If not all the pupae have hatched, wait until Thursday for this analysis.

PART 3: Work on your Module Projects

Fly Module Class 8. March 24, 2016

Work on Module Projects

No planned lecture.

IN THE LAB

PART 1. Finish screening any vials that were not read on Tuesday from your recombinant chromosome experiment and/or your RNAi experiment.

Part 2. Work on your Module projects

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Fly Module Class 9. March 28, 2016

Student presentations

Videos, Posters, Wikipedia, How-to-Instruction Manuals, Songs, Other!

2016

JANUARY

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