Welcome to the Lab!

1. Become Safe!

Follow the instructions for the EHS001 course on the Lab Procedures/Safety page of the lab website.

2. Lab Safety

Disposal of Glass:

***Non-hazardous*: Dispose of in cardboard glass box under bench**

***Hazardous with only residual*: For example, used formaldehyde ampules. After use, let them vent in the hood. After they are dried, place in the plastic box or jar labeled "Venting Glass Waste". After these plastic boxes are filled, the plastic box is to be taped up thrown in the dumpster**

Disposal of Tips and Tubes:

***Non-hazardous*: Non-hazardous tips and tubes from use on the benchtop are collected in beakers around the lab. This includes pipet tips, eppie tubes, falcon tubes and serological pipets. These are not to be thrown in the normal trash. After a beaker is full, dump it into one of the cardboard boxes under the bench next to the glass box. After full, tape this box up and throw it in the dumpster.**

***Hazardous (Not EtBr)*: Hazardous tips and tubes used in the hood, such as those containing TRACE amounts of phenol and chloroform, do not need to be entered into the hazardous solid waste stream unless what is being pipetted is highly toxic (ie, Sodium Azide). Let the tips and tubes vent in the "Venting Solids" container. After the container is full and sufficiently vented and dried, pour the tips into a gallon Ziploc bag (under the hood). Tape up bag securely and double bag if necessary. These bags are brought down and thrown in the dumpster.**

Ethidium Bromide:

Add EtBr to agarose solutions after cooling down sufficiently. Add at 10,000X with 10mg/ml EtBr. We don't stain gels separately so as to avoid sloshing around EtBR buffers.

Don't microwave solutions with EtBr.

Gels: **EtBr contaminated gels are dried down in the Tupperware lined with Saran Wrap in the hood. After drying down, they are placed in a Ziploc bag in the 5 gallon bucket underneath the gel bench.**

Tips: **Tips that were used to pipet EtBr directly (not gel loading tips) are collected in the glass jar. When the glass jar is full, dump it into one of the Ziploc bags into the 5 gallon bucket underneath the gel bench. Or, start a new Ziploc bag in the 5 gallon bucket.**

Buffers: **Buffers can be reused. After running a gel, if the buffer is going to be reused, poor it into the re-use buffer flask. If the buffer is getting nasty, it is time to dispose of it. Pour it into the EtBr "Tea" jar in the hood. This "Tea" Jar decontaminates EtBr and holds 3 Liters of standard 0.5 ug/ml Buffer. The tea bag can be used for 9 Liters of standard 0.5 ug/ml Buffer and the jar thus can be used for three fills. After filled up and left overnight, the buffer can be poured down the drain. Replace tea bag (throw in EtBr waste) after three fills.**

Fly waste/Biohazard waste:

All fly waste and biohazard waste are to be disposed of in Biohazard bags. After filling, they are autoclaved, put in a black plastic bag and thrown in the dumpster.

2. Lab Jobs

Washing Dishes- dish washing is the responsibility of the undergraduate student workers. All lab members will rinse their dishes and place in dish tub (with detergent) for soaking until wash time. Every day upon entering the lab, the assigned student will check dish tub and wash dishes if necessary. For washing use brush if right size and brush with soapy water from the tub. Rinse 2x with regular water and 2x with distilled water. Allow to dry. As soon as dry, cover with foil, add autoclave tape and place in autoclave tub to be sterilized. You can use the dry materials setting for glassware sterilization. All glassware and plasticware used in the lab should be sterilized for RNA use.

Making fly food- student workers will be responsible for making fly food. Please see fly food instructions. Fly food will be made every other week unless requested.

****The tasks of washing dishes and making fly food will be equally assigned by rotating lab duties every week.**

<u>Ordering</u>-all ordering will be completed by Michelle. Please send her an email request with complete information, mnewton@ku.edu, if you need an item. If you receive an item in the mail you will need to place the packing slip in her tray stacked under the lab printer. She will then make appropriate copies and update the lab spending workbook.

<u>Unpacking boxes/packing slips</u>- each lab member is to open/put away and turn in packing slip for items that they have ordered. If a lab member is absent and the package needs to be refrigerated then whoever receives the item is responsible for getting it into the refrigerator or freezer. Please leave a post-it note on Christine's computer if you put a package in the fridge or freezer. If you are not sure who has ordered a room temp. item, please leave the package on the counter by the door.

<u>Boxes/Autoclaving/Trash</u>- any lab member noticing a build-up of boxes or "to be" autoclave bags can take care of these items.

3. Lab protocols

<u>pH meter</u>: basic instructions posted on wall behind pH meter.

<u>Fly stock maintenance/Setting up Crosses</u>

Fly Work.

There are two major components to fly work: maintaining stocks, setting up/scoring crosses.

The peculiarities of fly work vary among species, so here is a rundown of the rules of the road for the two species groups we work with in the lab.

Note: The best way to maintain stocks is to use the lab identifier number on a tag with a plastiband *and* tape with the genotype/ID information on the vial. This way, when you are transferring, you carry the information forward with you and do not make writing necessary each time. However, when setting up crosses, etc, of course writing on the vial is easiest and sufficient.

See this as well:

http://flystocks.bio.indiana.edu/Fly_Work/culturing.htm

Fly Stock Maintenance.

For all species, when transferring, try to avoid overcrowding.

D. melanogaster and allies (simulans, sechellia, mauritiana and all other non-virilis species).

Maintain stock with plastiband and metal tag with stock number on it. Also on vial, write the genotype or identifying information on some tape and carry this tape across transfers. This will help identify vials if you don't remember what the number refers to.

The generation time of *mel* species is 10 days, so these fly stocks should be transferred every two weeks. Usually, you will transfer from the newer vial since this one will be two weeks old and should have flies that are nice and young having just eclosed. Always keep an old vial/bottle and a new one. This way, no vial or bottle is older than one month. Write the date on the vials when transferring/maintaining small batches. When transferring or maintain large numbers (in tubs) then keep track of transfer dates on the tub.

To avoid overcrowding, avoid putting more than 30 or 40 flies in a vial or more than 100 flies in a bottle.

D. virilis and allies (Americana)

Maintain stock with plastiband and metal tag with stock number on it. Also on vial, write the genotype or identifying information on some tape and carry this tape across transfers. This will help identify vials if you don't remember what the number refers to.

The generation time of *virilis* species is 18 days, thus, they are transferred every three weeks. Usually, you will transfer from the newer vial since this one will be three weeks old and should have flies that are nice and young having just eclosed. Always keep an old vial/bottle and a new one. This way, no vial or bottle is older than a month and a half. Write the date on the vials when transferring/maintaining small batches. When transferring or maintain large numbers (in tubs) then keep track of transfer dates on the tub.

To avoid overcrowding, avoid putting more than 10 to 20 flies in a vial or more than 50 flies in a bottle. For stock 160, however, the numbers can be 4 times higher per vial or bottle

Coddling stocks:

Some stocks are hard to maintain. For such stocks, it is often helpful to shove ¼ of a kimwipe into the food of new vials and a whole kimwipe into the food of new bottles. Watch these stocks closely and transfer faster than every two weeks if the adults are on food that appears dried up or nasty.

Vials or Bottles? For stocks you use frequently, it is a good idea to expand the stocks into bottles but keep a vial stock as back up. The bottle stock will be your working stock – maintain as many as necessary. The vial will be your back up stock.

Throw away old vials and bottles when they are expired in the biohazard bags and autoclave biohazard bags when full.

Setting up crosses.

The key to setting up crosses is to make sure you use virgins.

D. melanogaster and allies (simulans, sechellia, mauritiana and all other non-virilis species).

Virgin Collection:

These species mate 8 hours after eclosing. Thus, if you **completely** clear a vial or bottle, then come back less than 8 hours later, all the flies you collect will be virgins. Alternatively, female virgins can be identified by eye if they have just eclosed. They are almost white, quite distorted looking and have meconium in their gut. Of course, it doesn't matter if males are virgins for cross schemes.

Setting up a cross:

Two kinds of crosses can be set up: Single female or *en masse.*

Single female crosses are used if one is testing something about the genetics of the female, such as what her recombination rate is or how much non-disjunction she displays. For testing such things, do not use a female older than five days. In this case, use a single female virgin and three males. Put in the vial.

En masse: This type of cross can be done if progeny are needed to continue a crossing scheme. In this case, 3 to 5 virgin females can be placed in a vial with 3 to 5 males.

Brooding: In both cases, after adults have been on a vial for 5 days, brood them. Meaning, transfer the adults to a new vial for collecting additional progeny. Brood as needed, for how many flies are needed

Note: Since a fly generation time is 10 days, do not collect or score progeny from vials that are 9 days or older, since this could be the F2 generation

D. virilis and allies (Americana)

Virgin Collection:

These species remain virgins up to 5 days past eclosure. Thus, if you **completely** clear a vial or bottle, then come back 4 days later, all the flies you collect will be virgins. Alternatively, female virgins can be identified by eye if they have just eclosed. They are almost white, quite distorted looking and have meconium in their gut. Of course, it doesn't matter if males are virgins for cross schemes.

Setting up a cross:

If flies are less than 4 days old, they will not lay eggs for several days after setting up a cross since they are not sexually mature. Thus, if you set up a cross right away after collecting young flies, the first vials won't produce. Thus, often it is a good idea to age the flies an appropriate number of days before setting up a cross. Using flies that are 6 to 10 days old for crosses is ideal.

Two kinds of crosses can be set up: Single female or *en masse.* There are also some particulars of setting up dysgenic and non-dysgenic crosses.

Single female crosses are used if one is testing something about the genetics of the female, such as what her recombination rate is or how much non-disjunction she displays. For testing such things, do not use a female older than 15 days. In this case, use a single female virgin and three males.

En masse: This type of cross can be done if progeny are needed to continue a crossing scheme. In this case, 2 to 3 virgin females can be placed in a vial with 3 to 5 males.

Dysgenic and non-dysgenic crosses: Strain 160 is much weaker than strain 9. Thus, when setting up *en masse* **dysgenic crosses** in vials use 5 males of strain 160 and 1 or 2 females of strain 9. When setting up *en masse* **non-dysgenic** crosses, use 5 females of strain 160 and 3 males of strain 9. When one is testing either an aspect

of maternal repression or paternal induction of dysgenesis, it will be necessary to use only single females (maternal repression) or single males (induction). Ie, for testing maternal repression, one female with 3 males. Say for example you are interested in testing which chromosomes present in mom repress dysgenesis. In this case, use single females with the respective chromosomes.

Brooding dysgenic and non-dysgenic crosses: There is a significant effect of eclosion time on levels of gonadal atrophy. This means that in a crowded vial, the first 100 to eclose may have higher levels of atrophy than an uncrowded vial for which all progeny are counted. For this reason, to be consistent, two things must be considered. First, all progeny from a given cross should be counted. Second, to ensure this, it is important not to have crowded vials. Thus, for any scoring it is important to transfer adults **EVERY DAY FOR THREE DAYS.** For a given pair of parents, progeny should be counted from 3 vials, and all progeny per vial. Note: The earliest eclosers will always have higher levels of atrophy

Collecting dysgenic and non-dysgenic progeny: Leaving progeny on a vial for too long will cause progeny to die in the food. Thus, on the fourth day after progeny have started to eclose, collect the progeny and put on vials with yeast. Four days also ensures that no progeny will lay F2 progeny and the yeast will fatten them up for easy scoring. Collect progeny every four days until no more eclose. After progeny have been on yeasted vials for a couple days, begin scoring. This can be done according to a convenient schedule.

Note: Since a virilis fly generation time is 18 days, do not collect progeny from vials that are 32 days or older, since this could be the F2 generation

What goes where: Freezers/Enzymes etc.

When to use filter tips

RNAse free lab protocol