Introduction to working in the Ashworth *Drosophila* laboratories

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1.The lab- locations and where to keep flies

All fly storage and fly handling is carried out on **2nd floor in Ashworth 2**.
Below is a list of the rooms used for fly and bacterial work and a brief description of what each room is used for. **Not all flies can be handled/kept in all rooms**-

2.03- **Microbial culturing room**- *Cat 1&2 bacteria. No alive flies allowed*.

2.04- **Communal freezers, fridges and fly incubators***.* Fly storage but no fly work*. GM and non-GM flies, Cat 1 bacteria. Aliquots of Cat 2 bacteria are permitted to be stored on the labelled shelf in -70.*

2.05- **Constant temperature (CT) rooms**. Fly storage and bench space for general fly work/quarantine of GM flies. *GM and non-GM flies. Cat 1 bacteria.*

2.06- **Clean fly lab**. Bench space and microscope stations for fly work. *GM and non-GM flies. Cat 1 bacteria.*

2.07- **Fly infection lab**. Bench space and microscope stations for fly work. *GM and non-GM flies. Cat 1 & 2 bacteria. Viruses.*

2.02
Obbard bench space- Bench space and microscope station for work with wild-caught flies and non-gm quarantine flies. *Non-GM flies.*
Vale bench space- Bench space for bacterial work (left bench) and behavioural fly work (right bench). *Non-GM flies. Cat 1 & 2 bacteria.*

2.18 Stockroom- contains stock to replenish fly lab spaces. *No alive flies allowed*.

Incubators

For keeping flies-
The GM rules above apply to fly storage.

2.04- 25° clean incubator for clean flies
2.04 -25° bacteria incubator bacterial infected flies.
2.05- 18° CT room for clean flies.
2.05- 25° CT room for clean flies.
2.06- 25° clean incubator for clean flies.
2.07- 25° virus/bacteria incubator for virally or bacterial infected flies.

All fly incubators are kept at 12:12 h light/dark cycle. ~8am-8pm and each contain a tub of water to keep a nice humidity (~60-80%).

Note: Incubators must be changed manually when the clocks change. The CT rooms light cycle is control by the building staff and cannot be changed manually. Ensure that this is requested so all incubators are maintained to 8am-8pm.

2.07 - 37° bacteria incubator for plating bacteria cultures
2.10- 4° CT room for long-term storage of fly food and media (lewis, agar, etc.).

Molecular work is carried out on the **1st floor of ashworth 2 in rm 1.52**. You will be allocated a bench space prior to carrying out any molecular work.

*Note: No GM flies should be used in 2.02 as this area has not been sealed. This means the quarantine of GM flies must be carried out on the bench space in **2.05** instead.

2. Working in the lab- general rules

a) Using the stations-

Labs 2.05, 2.06, 2.07 and the Obbard/Vale bench spaces in 2.02 are a communal resources and all workstations are available for use to everyone.

Microscope stations should be used for work requiring microscopes (e.g. sexing flies, egg counting, and systemic infection of flies). Routine tasks such as fly tipping should be carried out on open workbench space if possible to free up microscopes.

If microscope availability is limited a booking system is available and it is possible to book microscope time using the sign-up sheet placed next to each microscope.

b) Equipment-

In each of the three main labs (2.02, 2.06 & 2.07) you will find the equipment necessary for general fly work. **It is important that equipment does not get moved between labs and that you replace equipment in the right place so the next person who needs it can find it.**

<u>i) 2.06 & 2.07</u>

There are labelled drawers containing everything you should need for fly work and general lab work including waste bags, pipette tips, microcentrifuge tubes, petri dishes, mats, funnels, etc. You will also find next to each microscope a tub containing tweezers, paint brushes, marker pens, etc.

<u>ii) Vale bench in 2.02</u>

Bacterial work is done on the left bench and here you should find everything you need (media ingredients, petri dishes, 50mL falcon tubes, etc.). This bench is for gloved hands only! You will also find autoclaved media and reagents sitting on this bench and an autoclave waste area.

Behavioural work is done on the right bench and here you will find the flyPAD, D.A.M, and behavioural arena equipment. There are pipettes and tips available on the behavioural bench which do not require gloves.

iii) If equipment runs out

There is a stock sheet available (attached at end of document and taped on the wall each lab) which tells you where you can replenish lab equipment from.

If the item you require is not on the list or cannot be found in the designated location either order it yourself directly through P&M or speak to Katy/appropriate PI.

iv) Trays for fly storage

Opposite the CT rooms in 2.05 you will find a cupboard and shelf space containing cardboard, plastic and metal trays for storing fly vials and bottles.

When you are finished with your tray it should be cleaned well with ethanol or frozen for ~24hr before you place it back in this cupboard.

Freezer space for this can be found in the Vale bay 2.02 and in 2.04.

c) Noise-

Fly tipping can be a noisy process! Please use the available mats/pillowcases to reduce the sound of 'tapping down' vials and bottles.

d) Cleaning up after yourself-

As the labs are often busy, it is **essential to clean up after yourself**!

Once finished at a work station clean the bench, microscope and the equipment you used with 70% ethanol and put all equipment back in its allocated drawer/shelf/etc. Aim to leave the workstation cleaner than you found it!

Microscope lenses can be cleaned using ethanol and lens tissue (kimwipe).

The sink space in 2.06 and outside the 2.07 door must be kept tidy – do not leave things in the sink for days and remove items from the drying area when dry and return to the correct place.

Chemgene and **bleach** can be found next to the sink in 2.06 for disinfecting bacteria and virus contaminated equipment. Please read and sign the COSHH form before using Chemgene and follow the dilution instructions on the label.

70% ethanol and **ionised H_20** bottles are available within each lab area. Please refill these if low. The 100% ethanol is stored in the chemicals cupboard at the end bay of 2.02 and the ionised water is above the chemicals cupboard (1x ionised left tank/3x ionised middle tank)

e) Hazardous spills

A **spill kit** is available under the sink in 2.06 and under the desk in 2.07 for cleaning up any spills. The building also has larger spill kits available in ash 3 foyer area.

If you spill a hazardous substance please follow instructions in the appropriate COSHH form which you should read prior to handling the substance.

If required there is a bag of vermiculite under the 2.06 sink for hazardous spills.

f) Disposing of sharps

A **sharps bin** is available on the workbench in 2.07 for disposal of sharps and small glass breakages.

If you smash something larger, the necessary clean up items can be found in the spill kit and a green wheelie bin for lab glass can be found next to the ash 3 lift.

3. Fly culturing

Please label any trays and/or individual bottles/vials clearly with

- **your name**
- **type of fly** (e.g. Oregon R wol+, 370 DGRP)
- **infection status** if appropriate (e.g. *Pseudomonas entomophila infected*)
- the **date flies were placed in vial/bottle**. Note: where you have a number of vials/bottles from different dates ensure that date of the oldest vial/bottle is marked on the tray.

Flies stored at 25° should be tipped onto fresh food, and the old cultures discarded, at least every 20 days. Flies stored at 18° should be tipped onto fresh food, and the old cultures discarded, at least every 40 days.

Failure to regularly tip your flies can lead to mite infestations (see **16. Mites**). We implement a rota'd fly monitoring system whereby every week a group member will check all fly stocks to ensure flies are being tipped within the appropriate time frame and that trays are labelled appropriately. Failure to comply will lead to naming and shaming via group email! If the same tray is mentioned 2 weeks in a row the tray will be removed from the incubator and placed in the 'fly sin bin' in 2.04.

4. Ordering fly food

Fly food can be ordered by emailing the media team (Anglea Reid and Alison Fulton) – ashworthmedia@ed.ac.uk

Fly food should be ordered at least 1 week in advance. If you require food sooner go speak to Angela directly to check if possible.
When ordering food you should state-
-your name and your PI
-the type of food you require (lewis bottle, agar vial, etc.)
-number of bottles/vials
-the date you require the food
On the date you requested your food, Angela will place any food ordered in 4˚ rm. This is where you should keep your food for long term storage (up to 3 months but ideally lewis/sya food should not be used for experiments if more than 3 or 4 weeks old).

Note: fly food stored at 4˚ must be brought to room temperature before use- flies paralyse at low temperatures and will stick to the food if cold.

5. Surplus food bottles and vials

If you have left over fly food bottles/vials you do not require please cross out your name and write 'free' on the tray and place back in the 4° room.
Before ordering food please check for 'free' food bottles/vials in the 4° room. Re-label any trays you wish to use with your name.

6. CO_2 anaesthetisation

Each microscope station has both a CO_2 gun and pad. Please ask to be shown how to use the CO_2 for *Drosophila* anaesthetisation. Ensure you are using a gun and pad which are connected to the same CO_2valve and that the pad is the correct way up. **Always** turn off the CO_2 at both the valve and main switch when not in use.

The CO_2 pads are made of porous polythene. This membrane is easily damaged so avoid banging vials or bottles on the surface. The pads are very efficient and a low flow of $CO_2$is sufficient to keep flies asleep (4-6 seems good). Once you have knocked out your flies, turn the CO_2 down to the minimum flow necessary to stop the flies waking up (you will see them twitching if they are starting to wake).
To avoid contamination risk, sellotape or elastic band a Kimwipe around the pad before placing flies on the pad. Remove and dispose of the Kimwipe when finished.

Note: flies dehydrate and die if left on $CO_2$for too long. Reducing the CO_2flow to the minimum necessary will increase the time you can leave the flies on the pad without them dying. Avoid leaving flies on a CO_2 pad for more than 20mins.

7. Waste disposal

There are various bins in each lab. These bins are specific in what can be put in them and waste should not just be thrown into the closest bin! If you are in doubt about how to dispose of something ask someone else in the lab or refer to the waste disposal diagrams (in 2.02, 2.06 & 2.07).

a) Non-lab waste
Within 2.02, 2.06 and 2.07 you will find a flip top bin next to the sink. These bins are for disposal of non-lab materials only (i.e. hand towels) and will be removed and replaced by the cleaners.

b) Recyclable waste
There are various recycle points throughout the uni for recyclable non-lab waste. For *non-contaminated* lab waste that is recyclable –

Small cardboard (i.e. tissue roll, glove box) in the blue/orange lidded recycle points in corridor
Large cardboard boxes should be broken down and placed in the large, blue bucket trolleys next to ash 2/ash 3 lifts
PP1-5 plastic ware (i.e. empty non-toxic reagent and chemical tubs, 96-well plate packaging) in the red swing-top bin opposite ash 2 lift (note: this bin only gets emptied if taken to the loading bay on a Thursday for collection Friday morning – it needs to be collected from here once empty)

Tip boxes – greiner and starlab tip boxes can be sent back to producer for reuse. Place tip boxes and racks in the appropriate collection points (starlab – green, material container next to water dispenser in 2.02/ greiner – cardboard box in 2.06 and/or 1.52). Other branded tip boxes can be placed in red plastic recycling bin if PP1-5.
Glass – green, wheelie bin next to ash 3 lift. If the glassware contained a toxic/hazardous substance then dry out in fume hood prior until all liquid has evaporated (use tissue wick to speed up process).

c) Non-GM/uncontaminated fly & lab waste

Within 2.02, 2.06 and 2.07 there are yellow bag bins; labelled **'Non-GM fly & lab waste'**. These bins are for the disposal of all fly and lab waste which is **NOT** GM or contaminated with biological or pathogenic agents.
When a yellow bag is full please tie-up, label (lab group, room number and date) and freeze for 24 hours (freezer space available in 2.02 or 2.04) before taking to Ashworth Stores for incineration. Place the bag outside stores and let them know you've placed it there – only take bags over when stores is open between 9-5pm. Remember to place a new yellow bag in the bin (drawer labelled 'refuse bags').

d) Autoclavable waste

In the 2.06, 2.07 and the Vale bench in 2.02 there are clear autoclavable bin bags. The autoclave bins are for the disposal of active GMM/GMOs, Biological Agents & Pathogens. Thus all **GM fly & bacterial contaminated waste** must be disposed of in these bags. These bags can't be too heavy, so when around 1/2 full these bins should be securely sealed with tape, labelled ('GM and/or bacterial waste', lab group, and room number) and placed in the black tub labelled '**autoclave bags**' in 2.06 or the equivalent green tub in 2.07. The media team will remove these bags for autoclaving and then dispose of them appropriately.
Remember to place a new autoclave bag in the bin (drawer labelled 'refuse bags').

e) Polystyrene

Place in the black bin bags provided in the loading bay on the shelf next to the metal cages.

8. Fly traps

Within the lab areas there are fly traps to catch escapee flies- a lewis food bottle with a cardboard funnel secured around the bottle opening. These are much more effective at attracting flies if you add a bit of dry yeast and water to the media surface.
The lewis food traps do not kill the flies and so must be replaced regularly. If you see a lewis fly trap which has developing pupae or is full of flies please dispose of (freeze then chuck in autoclave bag) and replace the trap using your own bottle or 'free' bottle.

9. Fly morgues

Fly morgues are simply a plastic bottle containing a small amount of water mixed with washing up liquid or ethanol (approx. 50:50 ratio) with a funnel on top. Use these morgues when you need to dispose of flies but retain the vial/bottle you are removing them from. When a morgue is full pour the contents into a freezer bag (in drawer labelled 'refuse bags') and freeze before disposal. Clean and refill the morgue ready for the next person.

Note: morgues get VERY stinky if not regularly replaced!

10. Lab coats

a) Where do I find a lab coat?
Lab coats can be found in the plastic bag labelled '**spare, clean lab coats**' under the behavioural bench in the Vale bay 2.02. Please help yourself to a lab coat - label it clearly with your name and lab group in permanent marker pen. If there is not a suitably sized lab coat available then please speak to your supervisor about buying a new one from stores.

b) How do I get my lab coat cleaned?

Make sure the lab coat is labelled in permanent marker with your name and lab group. Leave it on the top shelf of the trolley outside the media room (G.02).
The lab coats get collected on a Tuesday morning and are returned the following Tuesday, so it's best to drop them off on a Monday afternoon for the quickest turnaround. Sometimes they aren't

returned for 2 weeks so make sure you have a spare lab coat to use. Either Angela will return your coat to your lab or you can collect it from where you dropped it off.

If you are leaving the lab for good then please place your no longer required lab coat in the plastic tub labelled '**dirty lab coats**'.

11. Autoclaving

a) How do I get something autoclaved?
Either you can get the media staff to autoclave items for you or you can use the autoclave machine in 2.02.
It's really up to you what you prefer to do -there are benefits/drawbacks to both. Media only runs the autoclave on certain days and charge for use but glass and plastic ware will be dried after sterilisation so it's particularly useful for containers of eppendorfs/tips/etc. The machine in 2.02 is slower but free to use. If you use the machine in 2.02 you must sign up to the cleaning rota which means once or twice a year you will need to empty and clean out the machine.

Media autoclaving
Media staff run autoclave cycles on Tuesday and Thursday mornings – place items in G.01 before 11am on these days. The item must be labelled using autoclave tape (name, group, room number you want item returned to)**.** The media staff will return this item to the location you stated when ready. This will be the afternoon of the same day for liquid items (i.e. agar) or a few days later if its glass or plastic ware which requires drying in warming cabinet first.

If you have items which do not need to be autoclaved in a hurry (i.e bottles containing contaminated broth, etc.) then these can be placed in the autoclave tray on the bacterial bench in 2.02. Label the items as above. If the tray/area is full when you add your item then please take everything down to G.01.

Autoclave machine in 2.02
The instructions for use of the machine are on the wall behind the machine. You will find the cleaning rota to sign up to here, also. If you do not need to fill both baskets then its good etiquette to ask around 2.02 if anyone else needs stuff added or instead add some of the communal fly glassware if the 'autoclaved glassware' shelf is low.

b) Where can I find autoclaved equipment?

i) Glassware

In 2.06 above/next to the sink you will find ‘Clean glassware’ and ‘Autoclaved glassware’. Please help yourself. When you are done with the glassware, clean and replace (ensuring you remove any old autoclave tape as to not cause confusion as to what is autoclaved and what is not) or autoclave through one of the above methods.

ii) Plastics

Generally plastics (i.e. microcentrifuge tubes, pestels) are autoclaved on a need only bases by the person who requires them. These can be stored on the appropriate shelf space on the bacterial bench (labelled with your name). However, some of the autoclaved plastics here are communal so it is worth checking if you can use and replace something if you need it asap.

12. Risk Assessment and GM folders

Risk assessments, COSHH forms, safety data sheets (SDS) and the GM fly record folders can be found in **2.06** on the shelf above sink.

RA/COSHH forms

All persons must read and sign the appropriate risk assessment/COSHH forms prior to carrying out the work detailed within. If any of the work you intend to carry out is not covered by the risk assessments/COSHH forms available for your group then please write your own or speak to your supervisor.

Safety Data Sheets

SDS are available for all chemicals used in the labs. If you are unsure of safe protocol for using a chemical please refer to the SDS, along with the appropriate risk assessment/COSHH form.

GM folders

Each group has a folder with a record of all GM flies in that group’s possession, their location, no. of bottles/vials and tipping schedule. These must be kept up to date for HSE requirements on keeping GMOs.

13. Protocols

Protocols are available for many of the procedures we carry out in the lab including how to make reagents (i.e. PIMs), carrying out molecular work (i.e. RNA extractions) and general fly work (i.e. tipping flies, systemic injection). A paper copy of these protocols in available in the **'Vale protocols' folder in 2.06**.

A number of these protocols are also available on **protocols.io**, a website providing open access to a repository of scientific methods and a collaborative research platform. You can access this via the following link https://www.protocols.io/groups/vale-lab
If you require an electronic copy of any of the protocols not available on protocols.io then speak to Katy or your supervisor.

14. Fly stock & primer list

A list of all fly stocks held within the Vale lab is available at:
https://docs.google.com/spreadsheets/d/1gqZ7EfZCCn9UEv2X5kaqERcjR36nOtFnLZGq-o3zfvA/edit#gid=1924654486

A list of all primers held within the Vale group is available at:
https://docs.google.com/spreadsheets/d/1uuS-Ghx6YE1fZwH7f5sAkdRH2Gv4_va1J518-DVmTeA/edit#gid=2036060696

15. Quarantine

New fly stocks brought into the department, from whatever source, must be quarantined before being used in the labs. Please quarantine all individuals in the Obbard bench space 2.02 (but refer to **1. the lab- locations** for quarantining of GM flies).
On the day of receiving flies, transfer any adults or the first flies to eclose into new vials. Retain the original vial(s) for ~3 weeks and periodically check for the presence of mites and/or mite eggs. Every 2 days for at least another 2 times continue to re-tip flies into new vials. Continue to monitor the new fly stock for a further two generations before the flies can be brought into the lab for experimental work. For full details on the quarantine procedure visit:
http://www.flyfacility.gen.cam.ac.uk/Flylab/mites
Please do not attempt to shortcut this procedure.

16. Mites and mold

Stocks should be checked regularly for mites, mold and other contaminants. Regularly tipping your fly cultures and cleaning surfaces with 70% ethanol will minimise the risk of acquiring mites or mold within your cultures.

Mold

If your stocks develop mold then tipping your stocks through agar vials containing a strip of Kimwipe doused in 0.5% propionic acid for 3 days (or 3 tips over ~ week) allows the flies to clean themselves on the propionic surface and works well for 'cleaning up' your fly stocks. Return flies to lewis/sya and check the following generation if mold is still present. If so, repeat process. A 1% solution can be used if problem persists.

Mites

For more information on mites; what they look like, how to avoid getting them and what to do if you do have mites please visit http://www.flyfacility.gen.cam.ac.uk/Flylab/mites
and watch the following video https://www.youtube.com/watch?v=PfSAAsrVANc

Briefly, if you do find mites in your lab stocks-
-let everyone else working in the labs know
-discard all non-essential stocks
-clean all potentially mitey surfaces with 70% ethanol and freeze any potentially infested equipment at -80° for 24+hrs
-quarantine affected stocks in Obbard bench space 2.02/2.05 bench space. Select 5-10 pairs of clean adults (check them under microscope for mites/mite eggs) and place in a clean food vial. Transfer these flies onto new food once every 2 days, at least 3 times. Discard the first 2 transfers; continue to monitor the rescued stock for at least 2 generations.

17. Allergies

A very small number of people who are exposed to *Drosophila* develop allergies. Consequently soon after starting work in the lab your supervisor should notify Occupational Health Unit (OHU)

and you will be emailed asking you to attend a health screening appointment. The appointment will be brief and is non-invasive- you need to fill out two questionnaires and blow into a tube. Appointments are every 6 months for the first year and once a year thereafter.
If this has not happened, speak to your supervisor or email OHU directly - Occupational.Health@ed.ac.uk

Please inform your supervisor if you have any existing allergies that might predispose you to a fly allergy. If you are concerned you are developing symptoms contact your supervisor.

Where to find Lab Equipment

Most lab equipment needed for fly work should be found within each lab space (2.06, 2.07 and 2.02) in labelled drawers. If the drawer is empty or you require something else please see below for storage location. Always restock each drawer/lab from the location below-

Item	Location
White roll	2.02 Vale cupboard
Gloves (XS, S, M)	2.02 Vale cupboard
Kim wipes	2.02 Vale cupboard (additional available 2.18)
Filtered tips (p1000, p200, p20, p10)	2.02 Vale cupboard (additional available 2.18)
Tips (p1000, p200, p10)	2.02 Vale cupboard (additional available 2.18)
10mL & 25 mL stripettes	2.18
0.5 mL Eppendorf tubes	2.18
1.5 mL Eppendorf tubes	2.18
90 mm Petri dishes	2.18
50/55mm Petri dishes	2.18
7 mL Bijou tubes	2.18
25 mL Bijou tubes	2.06 under bench
50 mL skirted/non-skirted Falcon tubes	2.18
96-Well flat/round bottom plates	2.18
24-Well flat bottom plates	2.18
1mL cuvettes for cell density reader	2.18
Various PCR plates	2.18
Empty fly bottles	2.18 or G.02
Empty fly vials	2.04, 2.05 or G.02
Non-absorbent cotton wool	Black bucket in 2.02 (additional available in 2.05 or G.02)

Absorbent cotton wool	**2.06 under bench**
Foam bungs for bottles	**Green bucket in 2.02 (additional available in 2.05 or G.02)**
Agar	**G.02**
Dry yeast	**2.18**
Brown Sugar	**2.18**
Autoclave and yellow waste bags	**2.18**

If the item is not within any of the locations listed then we do not have it! Please speak to Katy or order the item yourself through P&M. Most of these items are available for internal ordering from Ashworth stores.