# GUIDELINE
# RAISING DROSOPHILA MELANOGASTER IN THE LABORATORY

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## 1. PURPOSE

The following are guidelines for working with the dipteran Drosophila melanogaster. They detail experimental practices for the care, maintenance and handling of laboratory cultures, and have been prepared with the intention of assisting those who already work with this organism as well as newcomers to this model system.

## 2. REFERENCE SOURCES AND STANDARDS

http://flystocks.bio.indiana.edu
http://flybase.org
Lindsley and Zimm (1992), The genome of Drosophila melanogaster (Academic Press);
GL: The Drafting of Guidelines for Scientific Research Laboratories According to Quality Principles. Lacerra G, Digilio FA, Lanati A, Liguori GL. Edition 0, date 11.03.2013
Legislative Decree n. 206 of 12 April 2001
Consolidated Legislative Decree 81/08 and subsequent amendments

## 3. ACRONYMS AND SPECIAL TERMS

| **Acronym** | **Meaning** |
|---|---|
| $CO_2$ | Carbon dioxide |
| Dm | *Drosophila melanogaster* |
| Dm Lab | *Drosophila melanogaster* laboratory |
| F2 | second generation |
| qPMO | quality Project Management Open-Lab |
| RT | Room temperature |
| VQ | Valore Qualità |
| WP1 | Workpackage 1 |

## 4. GUIDELINE AREAS

### 4.1. MILIEU

### Organization

The Dm Lab must be adequate to ensure the proper handling, cultivation, and monitoring procedures for the cultures.
The area dedicated to the preparation of growth media must either be separate from the area dedicated to working with Drosophila or have the possibility of being functionally partitioned to minimize contaminations.
Workstations in the laboratory should be equipped with a stereo microscope, a cold light source, a plate with a porous membrane (such as the FlyStuff flypad) attached to a $CO_2$ tank with a device that permits the regulation of the flow of gas needed to anesthetize flies, and other items necessary for working with them.
To minimize the number of free flies within the Dm Lab, it is necessary to set traps, or glass jars closed with a plastic funnel and filled with wine vinegar and a few drops of liquid soap. This kind of trap is not recommended for use in climate-controlled rooms or incubators because of the corrosive effect of the vinegar. Instead, in these spaces, trays containing the cultures can be covered with layers of cotton gauze.
The workstations and materials are assigned by the Researcher-in-charge of the Dm Lab, whose decisions must be respected. Each unit of personnel in the Dm Laboratory is responsible for the management of their assigned workstation.

## Cleaning

The Dm Lab must be kept free of any kind of infestation through the proper application of preventive measures. To clean work surfaces, incubators, equipment, utensils, glassware and instruments, it is recommended to use: denatured alcohol and/or 70% ethanol. Any residue on the tray containing the cultures should be removed using a sponge soaked with 70% ethanol or denatured ethanol.

To clean the containers, it is good practice to freeze them if there are flies inside them. Then remove the food and autoclave them for 20 minutes at 121° C (if the containers are made of plastic, ensure that they can be autoclaved) or rinse them in a 10% bleach solution.

At the end of their activities, each unit of personnel is responsible for leaving their workstation clean and in order. All affiliated personnel, along with the technician-in-charge, must participate in the cleaning shifts for the growth room/incubator and the regular six-monthly cleaning of the laboratory, according to the schedule set by the technician-in-charge (Form 1).

### 4.2. MANPOWER

For the optimal management of the Dm Lab, it is helpful to assign specific tasks to specific individuals.

**Researcher-in-charge**

- He/she is the manager of the laboratory in which experiments on Drosophila are carried out.
- He/she selects the technician-in-charge and ensures the adequate training of all personnel affiliated with the Dm Lab.

**Technician-in-charge of the Dm collection**

- He/she is selected by the Researcher-in-charge of the Dm Lab.
- He/she is responsible for the care and maintenance of laboratory cultures, updating the list of strains, assisting researchers and trainees in experimental procedures, planning and overseeing the cleaning of the climate-controlled rooms/incubators and work surfaces, ensuring that devices and equipment are maintained, ordering materials and providing them to the Dm Lab personnel so that everyone may work under optimal conditions.
- He/she should be contacted whenever there are problems related to the cultures and/or equipment.

**Personnel affiliated wtih the Dm Lab**

- Researchers, technicians and trainees (students, fellows, graduate students, etc.) participating in research projects in the Dm Lab.

### 4.3. MACHINES

Climate-controlled rooms and/or stand-alone incubators with neon lights calibrated to recreate light-dark cycles (a cycle of 12 hours is recommended) and humidity of approximately 60%: incubators must be kept absolutely free of loose flies and be cleaned of fly carcasses and other contaminants. These devices should be equipped with a remote alarm or a switch which interrupts the electrical current in case of the accidental malfunction of temperature controls.

Stereo microscope with a distant cold light source. Before and after handling flies, the stereo microscope, work surface, $CO_2$-flypad apparatus and brushes should be cleaned with 70% ethanol or denatured ethanol.

Maintenance and care of equipment shall be carried out by the technician-in-charge, who may utilize internal or external services with the details, date, and signature recorded (Form 2).

## 4.4. MATERIALS

**Food-grade $CO_2$ tanks**: these must be placed in appropriate spaces outside the laboratory or, if placed within it, they must be anchored to the wall, according to the regulations.

**Materials for handling Drosophila melanogaster**: soft, fine-tipped watercolor-type paintbrushes, plate with a porous membrane, white cardboard-type surface, a "morgue" container (a jar filled with alcohol in which to deposit dead or anesthetized excess flies), containers for raising flies, glass jars, funnels, hydrophobic cotton wool, cotton gauze, etherizer, trays for storing cultures, thin and absorbent paper, glass and/or plastic Petri dishes, containers for ice upon which microscope slides can be placed, equipment needed for the preparation of growth medium (pot, automatic dispenser, polenta, yeast, agar, sugar, Nipagin).
Containers for growth medium are short glass or plastic cylinders, closed at one end, whose diameter can vary from 20-30 mm up to larger dimensions. For safety and convenience, it is recommended to use plastic containers which can be purchased from suppliers of Drosophila (FlyStuff) or laboratory equipment (Kaltek, Sarstedt).
Containers of larger dimensions are recommended for maintaining large fly populations, whereas for housing the strains of the laboratory's collections it is preferable to use smaller containers.
To reuse glass culture containers, it is important to completely clean and sterilize them, as indicated, to prevent contamination.
All containers can be closed with different plugs, but it is recommended to use hydrophobic cotton plugs which are not to be reused.
To avoid contamination between different strains, any cotton plug accidentally dropped on the floor should be thrown away and replaced with a new one.
For special growths, the cotton plug can be wrapped in pieces of hydrophilic gauze to keep overcrowded cultures as dry as possible.
Another expedient to reduce the humidity of overcrowded cultures is to insert small pieces of absorbent paper into the growth medium.

**Detergents**: Denatured alcohol, 70% alcohol, bleach diluted to at least 10% and special detergents.

## 4.5. METHODS

### 4.5.1. PREPARATION OF GROWTH MEDIUM

There are many nutrient media suitable for raising Drosophila melanogaster in the laboratory. The fundamental characteristics of a growth medium, regardless of the recipe chosen, are:

- the right consistency and hydration so that flies do not drown in it;
- the ability to inhibit the growh of molds, which are almost always present in the air and on flies.

A recipe and preparation procedures for growth medium can be found in the protocol: Preparation of growth medium for D. melanogaster. The correct consistency and hydration level of the medium must be calibrated experimentally because they depend on external variables, such as the level of humidity in the workplace. To adjust these parameters, it is possible to modify either the amount of agar used or the cooling/drying times for the medium. It is important to perform this last step under controlled conditions so as to prevent the entry of flies into the containers (Preparation of growth medium for *D. melanogaster*).

### 4.5.2. ANESTHETIZING FLIES

The following methods can be used to anesthetize adult flies:

- ether, not recommended because it is flammable, which has a penetrating odor and is capable of killing flies with an overdose. It is recommended for use in dissection of adults on slides;
- Flynap (brand of Carolina Biological), which has an unpleasant odor;
- $CO_2$ which, used in a continuous flow at the minimum level needed to anesthetize the flies, permits them to be immobilized for a few minutes with no side effects;
- cooling, a simple method recommended, above all, for behavioral studies because it does no neurological damage to the flies and which can also immobilize larvae.

Experimental practices suggested for each method are reported in the attached protocol: Anesthetizing *D. melanogaster*.

### 4.5.3. HOUSING OF STRAINS

The strains can be kept at RT, but must not be exposed to direct sunlight and/or air currents. It is best to keep the strains in climate-controlled rooms/incubators, under conditions of controlled temperature and humidity.

The name and date of inoculation should be written on each fly container. The name can be a number which corresponds to a list or, alternatively, the complete genotype of the strain.

It is important to have a list of the various strains in the collection which lists their genotypes, generic characteristics, and special needs; this list must be constantly updated by the technician-in-charge, under the supervision of the researcher-in-charge of the Dm Lab (Form 3).

Most strains can be maintained by periodically transferring adults to containers with fresh food, after checking them with a stereo microscope to ensure that both sexes are present and that their phenotype matches the one expected.

It is important to avoid overcrowded cultures, so it is recommended that about 20 flies be transferred into new containers.

The frequency of transfers depends on the housing temperature. The length of the *D. melanogaster* life cycle varies in relation to this parameter: it is 10 days at 25°C, and 21 days at 18°C. Strains should be refreshed every 14-16 days if kept at 25°C, or 25-30 days if kept at 18°C. They should never be kept for more than 30 days because older containers are more susceptible to infections.

The strains of the collection, which are not used daily, must be maintained in incubators or climate-controlled rooms at a temperature of 18°C. It is recommended to maintain at least two independent cultures for each strain, possibly staggered approximately 2 weeks apart.

It is good practice to keep the old cultures for 2 weeks at 18°C after the transfer so that they can be used as a spare if the new containers become damaged.

At each transfer, it is important to recopy all the indications regarding the type of fly onto the new container. Great care must be taken to avoid transcription errors.

Some strains require special selection at each generation. The technician-in-charge must be aware of this and report any special conditions for maintaining a certain strain on the Suggested form for listing strains (Form 3).

The technician-in-charge of the fly collection replicates strains upon request of Dm Lab personnel. The replicates are handled by a technician who stores them on trays which are different from those of the collection and affixes a unique identification code to them. The replicates must not be mixed in with the collection strains.

It is advised to have a "hospital tray" among the strains, a place to store strains which are sick or in crisis and need special attention.

#### 4.5.4. COLLECTING FEMALE VIRGINS

In a genetic cross for which it is necessary to know both parents' genotypes, it is necessary to use females that have never mated, termed virgins. *Drosophila melanogaster* females store the sperm cells from matings in their abdomens, which fertilize a large number of eggs. Since females do not mate in the first 10-12 hours after hatching from the pupal case at 18°C, virgins can easily be collected by the attached protocol: Collecting *D. Melanogaster* virgins.

It is important that for each collection the females be put in separate containers which should be checked for the presence of larvae after 2-3 days: if any are found, the females are not usable. Bear in mind that virgin females do lay eggs, albeit in reduced numbers, so it is the presence of larvae that indicates the flies are not virgins.

It is important to collect more virgins than necessary in case of any errors and/or fly deaths.

If a collection is missed, or only a few virgins are needed for one or a few specific crosses, virgin females can be identified by the presence of a dark spot in the abdominal region (Collecting *D. Melanogaster* virgins).

#### 4.5.5. SETTING UP CROSSES

It is impossible to give universal rules for setting up or scoring crosses as the precise protocol will vary from experiment to experiment.

Although crosses can be set up with a single pair of parents, using 3-5 females and 2-5 males per small container (about 20 mm in diameter), or 30 females and 10-20 males per jar (about 50 mm in diameter), is recommended for the greatest probability of success.

To maximize the number of progeny, crosses should be duplicated every 2-4 days, transferring the parents to containers with fresh growth medium with a small amount of brewer's yeast paste on the surface (optional but recommended).

Offspring should be scored at least once a day for at least 9 days from the first emergence of progeny since many genotypes have a developmental delay and/or a different life cycle. It is important to stop scoring the progeny after 9 days at 25°C because F2 will begin to emerge.

When setting up crosses, special attention must be paid to recording the dates and results of the crosses, comments regarding techniques, and results of the experiments on appropriate laboratory forms.

#### 4.5.6. GENETIC NOMENCLATURE FOR DROSOPHILA MELANOGASTER

To ensure a systematic and nonredundant nomenclature, it is recommended to use the rules adopted by Flybase.

Names of genes are usually written in italics.

The name begins with a lowercase letter if the mutant phenotype is recessive with respect to the wild-type in a normal diploid. The name begins with a capital letter if the mutant phenotype is dominant with respect to the wild-type in a normal diploid. Genes named after a protein product or other molecular feature begin with a lowercase letter.

Chromosome 1 refers to the sex chromosome and is usually indicated by an X.

The convention for writing genotypes is X/Y; 2$^{nd}$/2$^{nd}$; 3$^{rd}$/3$^{rd}$ (e.g., y,w;Cy/If;Sb/+).

Wild-type alleles are indicated by a + symbol.

It is recommended to consult Lindsley and Zimm (1992), The genome of Drosophila melanogaster, or the Flybase website for a detailed description of balancer chromosomes and many mutant alleles.

### 4.5.7. Waste

Fly containers are disposed of in special waste receptacles furnished by the Health and Safety Office and do not need to be autoclaved.

All Dm Lab personnel are required to properly close the solid waste container well when it is full. In turns, it is to be put out on the waste collection day (Form 4).

Glass and sharp waste are collected in specific containers provided by the Health and Safety Office. Glass must not overflow them and, once they are emptied, they are closed and put inside the solid waste container.

### 4.5.8. Contaminations

The most common contaminants affecting *D. melanogaster* cultures are bacteria and molds on the growth medium, and mites.

#### 4.5.8.1. Bacteria

The most common bacterial problem is bacteria that produce mucus on the food, in addition to a possible reddish-brown color (e.g., Acinetobacter sp.). Remedies can be found in the attached protocol: Remedies for contaminations of *D. melanogaster* cultures.

#### 4.5.8.2. Molds

Molds, above all species of the genera Penicillium and Aspergillus, are a common problem because fly growth medium is an ideal substrate for their growth. When strains are healthy, flies naturally compete with fungi. The problem arises with sick, weak or low-density cultures. It is good practice to follow the protocol: Remedies for contaminations of *D. melanogaster*.

#### 4.5.8.3. Mites

The most serious threats to *Drosophila melanogaster* cultures are mite infestations. The best preventive measures against mites (detailed in the attached protocol: Remedies for contaminations of *D. melanogaster*) are:

- to quarantine strains arriving from outside laboratories and/or stock centers;
- regularly refresh laboratory strains;
- follow recommended cleaning procedures.

In case of a mite infestation, it is necessary to:

- inform the technician-in-charge;
- dispose of contaminated containers outside of the Dm Lab;
- clean anything that has come into contact with the infected container according to the procedures specified in the cleaning section (4.1, 4.3);
- check all containers located near the contaminated container for mites.

If it is necessary to save a mite-infested strain, select 2-3 females and 1-2 males from the contaminated culture, make sure that their bodies do not have mites or their eggs on them, and place them in a new container in quarantine.

## 5. FORMS

Form 1 Dm Lab cleaning shifts

Form 2 Dm Lab equipment maintenance and care

Form 3 Suggested form for Dm Lab listing strains

Form 4 Dm Lab waste removal

## 6. SUPPORT SERVICES

**Health and Safety Office** **Person in charge ………………….. Telephone** …………………

For solid waste containers, sharp waste containers, and liquid waste tanks.

**Technical Support Services** **Person in charge ………………….. Telephone** ………………….

For climate-controlled rooms/incubators, $CO_2$ systems.

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## 8. ACCOMPANYING DOCUMENTATION

Preparation of growth medium for D. *melanogaster*

Anesthetizing *D. melanogaster*

Collecting D. *melanogaster* virgins

Remedies for contaminations of *D. melanogaster* cultures