Raising *Drosophila melanogaster* in the laboratory

Anesthetizing *D. melanogaster*

Experimental procedures for using ether, Flynap, CO_2 and cooling.

Ether: to anesthetize flies with ether, they are gathered in a cylindrical glass container (etherizer) with a funnel inserted into the open end. Gently knock the flies to the bottom of their storage container by tapping it on a soft surface such as a mousepad. The flies will stay on the bottom for a few seconds, long enough to quickly remove the plug, invert the container over the funnel and gently tap the two containers together to make the flies fall into the etherizer. The funnel will prevent them from getting out of the container. When all of the flies have been gathered, knock them down by gently tapping the etherizer on the mousepad and quickly replace the funnel with an ether-soaked cotton and gauze plug. Wait about 1 minute until the flies fall to the bottom and stop moving. Do not exceed the time or the flies will die.

To use **Flynap**, **CO_2**, or **cooling**, flies are anesthetized in their container. To avoid having the stunned flies fall onto the culture medium and possibly become stuck, it is important to invert the container so that they will end up on the cotton plug before administering the flynap, by inserting a cotton swab soaked in it into the container, or CO_2, through a Gilson tip properly attached to the system.

To incapacitate flies by **cooling**, containers are placed upside-down in the freezer until the flies stop moving, approximately 8-12 minutes. After this time, collect the immobilized flies on a cold surface that can be constructed by filling the top of a Petri dish with crushed ice and covering it with the the bottom of a Petri dish.

To immobilize fly larvae, it is recommended to put them on slides resting on a Petri dish filled with crushed ice.