

Flash Freeze Fruit Flies in Liquid Nitrogen

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Abstract

A protocol for routine flash freezing of fruit fly samples prior to use in downstream protocols (e.g. RNA extraction).

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Guidelines

Freezing should be done for a standard age or age range and within a 2 hour standardized window. Many genes are regulated according to the circadian rhythms and we want to minimize expression variation that results from the time of day.

Before start

Make sure you have the following items: LN₂, dewar (~2L), a large rectangular Styrofoam tub, colored timex tape and permanent markers.

Materials

Drosophila Vials 75813-160 by [Vwr](#)

Externally Threaded Self-Standing Cryovials with Silicone Washer Seal Cap 10018-734 by [Vwr](#)

2 Cryogenic box, with drain slots, with grids, 81 cell divider 89214-752 by [Vwr](#)

Protocol

Fly Sample Collection

Step 1.

When virgins are collected count how many are placed into each vial and write this on the outside of the vial.

Fly Sample Collection

Step 2.

Check virgin collection vials for larvae.

Fly Sample Collection

Step 3.

If no larvae present, consolidate by E# (cross identifier) into a single vial and write total number of flies for all vials corresponding to progeny from that cross of that type on the vial.

One Day to One Week Before Freezing

Step 4.

Create a spreadsheet that will record the information for all your samples and where they will be located in the freezer box and in the -80 freezer.

One Day to One Week Before Freezing

Step 5.

Before starting, check virgin collection vials for virginity a second time.

One Day to One Week Before Freezing

Step 6.

Pre-label freezer box with Experiment Title, Block # (if there are multiple blocks), Date, your name and email.

One Day to One Week Before Freezing

Step 7.

Pre-label Cryovials with V# (identifier for order vials are frozen in, starting @ 1), E # (cross identifier), sex, genotype, block #, and date; add the # of flies when you actually freeze. This information comes from your spreadsheet. You can use shorthand- but it should be explained longhand in your spreadsheet.

Day of the Freeze Before Starting

Step 8.

Put tape on bench to note the time tubes should be frozen (we allow flies 15 minutes to relax after being pounded through the funnel into the Cryovial).

Day of the Freeze Before Starting

Step 9.

Get liquid Nitrogen before starting at 3 p.m. (or the time you have chosen ... this will be fixed for all your experiments) and put in a rectangular Styrofoam tub that is big enough to hold two freezer boxes. Remember all flies must be frozen in a 2 hour window.

Freezing

Step 10.

If you are freezing Cryovials of multiple genotypes, arrange vials so that no one factor is grouped by time (aka have a few of each genotype frozen at the same time). The easiest way to do this is to freeze all the vials that correspond to the first replicate, then the second replicate and so on.

Freezing

Step 11.

For large freezes, we generally have 2 or more people seated in front of the racks of vials and freeze them from left to right front to back (with each person putting the flies in the vials in their section into cryotubes).

Freezing

Step 12.

For freezes that do not use anesthetic- Select pre-labeled Cryovial and virgin vial, pound flies through funnel into Cryovial. Screw lid on loosely, but securely.

Freezing**Step 13.**

For freezes under CO₂, knock-out flies, count them on the pad and sweep into the Cryovial. Screw lid on loosely, but securely.

Freezing**Step 14.**

Place Cryovial with flies in it on tape and note the time (we usually do this by grouping cryovials in 5 minute blocks) and in approx. 15 minutes place the cryovials in order by V number from left to right front to back directly into the freezer box that is sitting in liquid nitrogen. Make sure to do this quickly and that the vial is submerged. You can use tweezers to submerge the vial first to the side of the box and then put it in the right position in the box.

Freezing**Step 15.**

After you freeze them you can use the tweezers to rearrange them so that they are logical in terms of replicates and types or leave them in the freeze order. Whichever you do, you must make sure that your spreadsheet records where each cryovial is located in the box (A1 is the first slot, row A, column 1).