Protocol: Thawing Frozen Cells

Thawing frozen cells from the DGRC's *Drosophila* cell culture collection

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Cells are shipped as frozen stocks on dry ice. You can move the ampoules to a -70° freezer or to liquid nitrogen upon their arrival, but **WE STRONGLY ADVISE THAT YOU THAW THEM IMMEDIATELY UPON ARRIVAL**, since changes in temperature of the frozen ampoules often leads to cell damage.

Each ampoule contains approximately 0.5 ml of a cell suspension (approximately 2×10^7 cells/ml) in M3+BPYE + 20% FCS + 10% DMSO. The ampoules were not necessarily upright when they were frozen, so be careful when you open them, making sure that the contents do not spill.

To thaw an ampoule of any Drosophila cell line:

1. Place 5 ml of an appropriate medium in a 25 cm^2 T-flask. Recommended media are described for each cell line on our website.

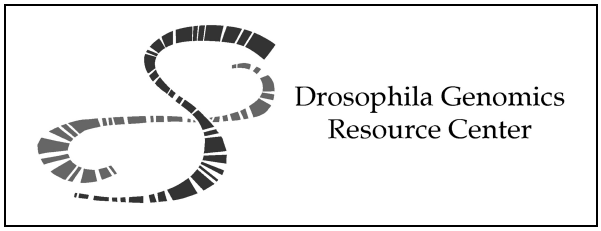
   NOTE: You may substitute a 60 mm tissue culture petri plate containing 4 ml of medium, but we do not recommend this for lines that are likely to grow slowly, because there may be substantial evaporation from the plate before the cells are ready for transfer.

2. Remove the ampoule from the dry ice. Using a Pasteur pipet, add a few drops of medium to the ampoule, and pipet up and down to thaw and mix the cells. Pipet the liquid back into the flask or plate, and repeat as necessary.

3. Place the flask of freshly thawed cells into an appropriate incubator for 1-2 hr (25° for most cell lines; lines requiring a different temperature are specified on the DGRC web-site).

4. Examine the culture in an inverted microscope. Usually, most of the cells are adhering loosely to the substrate at this point. If this is the case, gently remove the supernatant, and replace it with 5 ml of fresh medium. The purpose of this maneuver is to remove the DMSO, which is toxic to many lines. Leave the flask in the incubator overnight, and then repeat the change of medium.

   NOTE: Some lines, such as S2, are relatively insensitive to DMSO, but we find these changes of medium to be very helpful in recovering less hardy lines from frozen stocks.



NOTE: If the cells were mostly in suspension when you examined them after 1-2 hr in the incubator, you may find it preferable to use centrifugation to recover the floating cells: Remove the liquid from the flask to a centrifuge tube, and add fresh medium to the flask. Spin the fluid that you have removed and discard the supernatant. Resuspend the pellet (which will probably be nearly invisible) in about 1 ml of medium from the flask, and transfer the resulting suspension back into the flask.

5. Some cell lines may take considerable time to begin growing. If the cells are not yet ready to be transferred within a week, withdraw half the medium and replace it with fresh medium. Repeat this step as necessary.

WARNING: Freshly thawed cells are often accompanied by a substantial amount of debris resulting derived from cells damaged during storage and/or transit. The amount of debris will be much greater if you put the frozen ampoule at -80 or in liquid nitrogen rather than thawing it as soon as it arrives. The debris includes mitochondria, which look a lot like bacteria. Do NOT simply declare that the culture is contaminated and throw it out. Instead, watch the culture for a few days; if there actually is bacterial contamination, it will increase dramatically overnight. If the little black specks that you see are cellular debris, they will increase little if at all, and may even disappear over the course of a few days. If there is debris, but there are also healthy cells, the healthy cells should grow and take over the culture. After a few days, please notify us (dgrc@cgb.indiana.edu) if there is actual bacterial contamination, or if all of the cells are dead.