Lazzara Lab
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Thawing mammalian cells

Materials:

- complete media
- 10 cm plates
- 37ºC water bath

Procedure:

Note that it is generally best to thaw cells late in the day. This timing permits a media change (to remove DMSO from the freezing media) early the next morning after cells have been allowed to adhere to the dish overnight.

1. Prep the hood with your warmed complete media and one fresh 10 cm plate per vial you intend to thaw. Label your plates ahead of time to avoid confusion later.
2. Remove frozen vial of cells from the liquid nitrogen cell bank, and update the cell bank map accordingly. Place the vial in the -80ºC freezer if you are not quite ready to thaw it.
3. Thaw the vial in the water bath by holding just the bottom in the water and moving the vial back and forth. Avoid putting the vial so deep in the water that the cap touches the water. Check the vial every 10 sec or so to see if a layer of fluid has formed around the frozen plug of cells.
4. Once you can see a thin layer of fluid around the sides and on top of the frozen plug, remove the vial from the water bath and thoroughly drench it in 70% EtOH. Dry the vial well with a paper towel, and place it in the hood.
   Note: Use caution in cleaning the vial with EtOH because the label may wash off depending on the marker used to write the label. This can be especially problematic if you are thawing multiple vials at once. For this reason, and to avoid confusion, it is generally best to thaw one vial at a time.
5. Unscrew the cap of the vial and place it to the side. Lift the lid off the labeled 10 cm plate, and dump the frozen plug of cells into the 10 cm plate in one motion. If you find that the plug is still frozen at the top or sides, warm it between your fingers to the point that the plug releases from the tube.
6. Draw up 10 mL of complete media into a serological pipet and slowly drop the media onto the frozen plug of cells in the 10 cm dish to melt the plug and disperse the cells.
7. Mix the cells to distribute them evenly in the dish by rotating the dish back and forth, and place the dish into the incubator.
8. The next morning, check your cells to make sure they look good and are well adhered to the plate. Aspirate the media and put on a fresh 10 mL of warmed complete media.