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Thawing Cells Protocol

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Materials:

Cells (in this particular example, Human embryonic kidney 293T cells)
Media (see Ferreira Lab Making Media/Sterile Technique Protocol)
Drummond Original Pipet-Aid Pipette Controller (VWR, Cat. No. 53498-105)
Sterile 15 ml Falcon tubes (VWR, Cat. No. 89039-664)
Sterile 5 ml serological pipettes (VWR, Cat. No. 89130-896)
Sterile disposable plastic Pasteur pipette (VWR, Cat No. 10754-264)

Reagent/materials Setup:

- Set up a rack in front of you with labeled 15 ml Falcon tubes. Bring your media, cells, and a sterile 10 ml serological pipette into the tissue culture (TC) hood (*always open the media before opening the cells)
    a. Loosen the cap of the media and your Falcon tubes before opening your sterile pipette

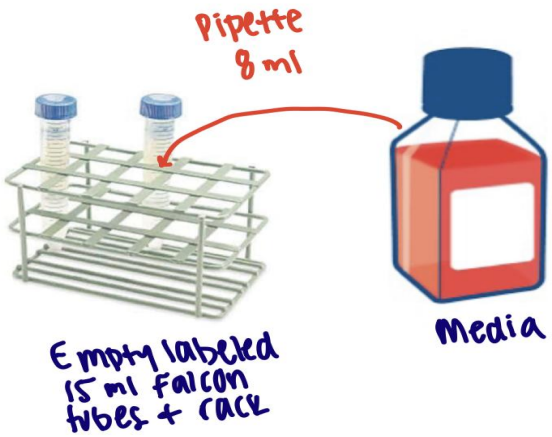

Figure 1. Experimental Setup of Reagents and Other Materials for Thawing Cells

Procedures:

1. Wipe down the hood with 70% ethanol (EtOH)
2. Label your 15 ml Falcon tubes with the cell line name, the date, passage number, and your initials
3. Pipette 8 ml of complete cell culture media (see Ferreira Lab **Making Media/Sterile Technique Protocol**) against the wall of a 15 ml Falcon tube using a sterile 5 ml serological pipette, *avoiding making bubbles - this will be your tube for washing the cells
    a. Remember: always open the media before the cells
    b. *Bubbles allow air to mix with the media, which cause you to lose cells so always avoid making bubbles
4. Quickly thaw your vial of cells in a heated, sterile water bath (autoclaved water) at 37°C for ~3 mins
5. Once the cells are thawed, sterilize the vial with 70% EtOH before bringing it into the TC hood
6. Then *immediately pour the cells directly into the Falcon tube containing media (no pipette necessary, but don't touch the rim)
    i. *DMSO is ideal for frozen cells, but toxic to cells at room temperature so they need to be put in media as soon as possible once they're thawed and 'awake'
    ii. This step and the wash step need to be completed swiftly
7. Washing the cells:
    a. Using a sterile Pasteur pipette, pipette enough media to wash the cryogenic vial the cells were frozen in (helps wash out any cells left behind in the vial; be careful still to not make bubbles)
    b. Add the media used to wash the vial containing the cells into the 15 ml Falcon tube

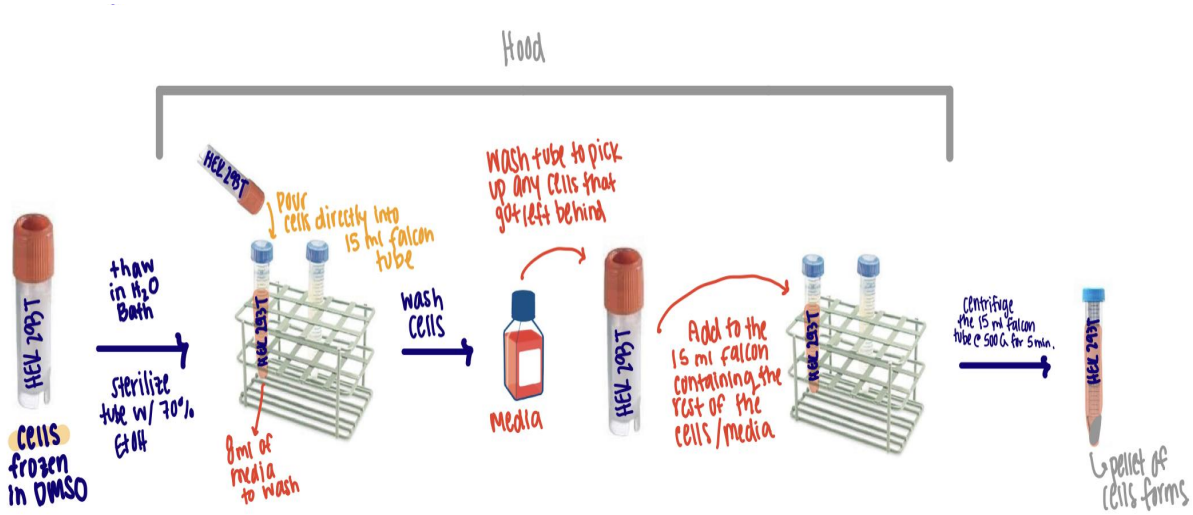

Figure 2. Workflow to Thaw a Cryogenic Vial of Frozen Cells

8. Immediately centrifuge the Falcon tube at 500 G for 5 min
9. After spinning the cells down to remove the DMSO, pour out the supernatant and resuspend the cells in medium to count the cells
10. Follow counting procedures and subsequent cell culture steps as described in Ferreira Lab **Adherent Cell Culture Protocol** or **Suspension Cell Culture Protocol** according to cell type

Supplemental Information:

- DMSO is ideal for freezing cells because it prevents the formation of water crystals, which perforate cell membranes and cause them to die. However, DMSO is toxic to living cells so as they thaw they are going to get 'mad' if you don't transfer them to media immediately