Thawing a Cell Line

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This protocol describes how to take a cell line from liquid nitrogen cryopreservation to culture. Give proper attention to good sterile technique at all times. It is written from a basis of approximately 1 million cells in 1 mL of freezing medium (see Cryopreservation protocol). Scale accordingly.

1. Prepare growth medium
    a. If full growth medium for the cell line of interest is not yet made, consult the subculture protocol and prepare the medium.
    b. Place 15 mL of full growth medium into a T75 culture flask (Corning 353110).
    c. Place 9 mL of full growth medium into a sterile 15 mL conical tube (Corning 352096). Place in a 37^{o}C water bath.
    d. Label the culture flask with your initials, the date, cell line and passage number of the cryopreserved vial.
    e. Place flask with medium into a 37^{o}C, humidified, 5% CO_2 incubator.
2. Retrieve, thaw, and prepare frozen stock
    a. Open the cryostorage vessel and retrieve the vial of interest.
        i. Be sure to take proper safety precautions with liquid nitrogen, which includes wearing protective gloves and ensuring robust ventilation. Close the cryostorage properly to make sure no gaps are present (minimize nitrogen leakage).
    b. Thaw stock by gentle immersion in a 37^{o}C water bath for ~1 min.
        i. Keep an eye on the stock because often it requires very little time for thawing.
    c. Spray cryovial and 15 mL conical tube from Step 1 with 70% EtOH and place them under the hood.
    d. Transfer the 1 mL of cell solution to the 15 mL conical tube.

    e. Spin according to subculture protocol (e.g. 100g, 3 min).
    f. Aspirate supernatant and resuspend cell pellet in 1 mL of full growth medium.
3. Seed cells
    a. Retrieve T75 flask from Step 1.
    b. Transfer the 1 mL of cell solution to the T75 flask.
    c. Close the flask and gently mix cells with medium.
    d. Return flask to incubator.
4. Update the cell stock information in Labguru.
    a. If you forget to do this I and/or others will find you and express discontent.
5. Replace growth medium the next day.
    a. Place full growth medium in a 37ºC water bath for ~10 min.
    b. If the line is adherent, check the cells under phase contrast for attachment. There should be a high proportion of attached cells relative to floating cells (>~80%).
        i. If there are many floating (i.e. dead) cells, use your judgement to determine the best course of action. One option is to get another vial and start over. Another option is to simply move on and let the living cells grow. Yet another is to lift the living cells and reseed them in a smaller culture vessel (e.g. T25).
    c. If the line is suspension, then take a small aliquot and estimate viability via trypan blue and a hemacytometer (see protocol for counting cells).
    d. Aspirate growth medium, wash once with 10 mL of room temperature 1X PBS (see solutions protocols), and replace with 10 mL of warm full growth medium.
    e. Place flask back in the incubator.