**Thawing a Cell Line**

*Walker Orr, 02/26/2024*

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| **Steps** |
| 1. Go to the QVEU liquid nitrogen inventory (Teams: QVEU\_LN) and identify cells you would like to thaw. |
| 1. Fill a conical vial with 9 mL of the appropriate media for your cells and place it in the water bath to warm. Also, make sure to have at least 5 mL of additional warm media for plating the cells. |
| 1. Get some dry ice and go to the liquid nitrogen freezer farm (in the building 33 basement). Ours is tank #8. |
| 1. It’s very important to wear cryoprotective gloves for all subsequent steps. Liquid nitrogen is very, very cold and can fuck you up almost instantly. |
| 1. Remove the foam plug from the liquid nitrogen tank (ours is tank #8). Press “start fill” and allow the tank to fill with nitrogen for around 10 seconds. You’ll see liquid nitrogen flow out of the tank. Press “stop fill” after this period. It’s tough to identify which liquid nitrogen box tower is ours. Go with someone who knows what they’re doing until we figure out a better system ☹. |
| 1. Slowly and carefully remove our rack tower from the tank. Working very quickly, but very carefully, remove the appropriate box from the tower. They’re numbered from the bottom up. Identify the cells you need, double-check, and transfer the correct vial to dry ice. Slowly and carefully replace the rack in the tank. |
| 1. Back upstairs, thaw your cells in the water bath, swirling gently to accelerate the thaw and avoiding touching the seam or the lid to the water. |
| 1. Spray the vial with 70% ethanol before opening. After that, pipette the cell volume into the prepared falcon tube (with 9 mL media) and centrifuge for 5 minutes at 125 x g. |
| 1. Remove supernatant without disturbing the pellet. Add 5 mL of DMEM and mix gently. Transfer that volume to a T-25 flask. |
| 1. Place in the appropriate incubator until the next day. |
| 1. Observe cell growth and expand when ready to a T75 and then maintain normally. |