Lazzara Lab
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Freezing mammalian cells

Materials:

- complete media
- FBS
- DMSO
- PBS
- 0.25% trypsin-EDTA
- centrifuge tubes (15 or 50 mL conicals)
- cell plates
- cyro vials
- cryo vial holder
- freezing container (“Mr. Frosty”) with isopropyl alcohol (IPA)

Procedure:

NOTE: DMSO is toxic to cells, though also necessary to prevent the crystallization of water during the freezing process (which subsequently lyses the cells you are attempting to freeze). Because of this, it is imperative to complete the freezing process quickly to prevent cell death.

1. For confluent plates, freeze down 2-3 vials per plate. For cell lines that are particularly slow-growing and/or do not grow to complete confluence, one vial per plate may produce better results on thawing.
2. Before trypsinizing cells, prepare everything needed for freezing procedure first:

    a. freezing media (1 mL per vial)
        i. 10% DMSO
        ii. 40% FBS
        iii. 50% complete media

    b. labeled freezing vials
        i. cell type
        ii. passage
        iii. initials
        iv. date

3. Aspirate media from cells, rinse with PBS, aspirate PBS, and add 1 mL trypsin-EDTA per 10 cm plate.
4. Once the cells are trypsinized, add complete media to plates and pool into conical tubes. Try to avoid trypsin to media volume ratios greater than about 1:4 when pooling cells. For example, for 2 plates with a combined total of 2 mL trypsin, add 8 mL of complete media to one plate, mix, and then add the resulting 9 mL to the next plate and mix. Place the resulting 10 mL pooled cell mixture in a conical tube.
5. Spin down tubes at 1000 rpm for 5 min.
6. Aspirate media and resuspend cells in freezing media, using 1 mL per frozen vial.
7. Add 1 mL of the cell/freezing media mixture media to each vial. If using Nalgene cryo vials, the Nalgene tube rack is a handy tool because it allows for one-handed opening and closing of the cryo tubes.
8. Tighten caps on vials and place them in freezing container with IPA. [Note that the IPA can only be used to freeze down cells 5 times before it needs to be replaced. Be sure to check off the hand-made IPA usage counter after each round of freezing.]
9. Place freezing container in the -80 freezer overnight, and transfer the frozen vials to the liquid nitrogen cell bank the following morning. Note the location of your newly deposited cells in your cell bank map, making note in the map of the same items listed in 2b above.