Freezing Immortalized Cells

Materials
Freezing media: 90% fetal bovine serum (FBS) + 10% DMSO
Cell media
Phosphate buffered saline (PBS, 1X, calcium- and magnesium-free)
Trypsin-EDTA (0.25% w/v)
Pre-labeled cryovials
Mr. Frosty container

Protocol

1. Aspirate cell media
2. Rinse 1X with PBS
    a. This removes traces of trypsin-inhibiting serum from the cells
3. Add trypsin. Incubate 5min. Image. If cells have not yet lifted off the plate, incubate 5-10min more. Do not exceed 15min in trypsin. Scrape cells if necessary.
4. Add an equal volume of cell growth media containing serum.
    a. Serum stops the trypsin reaction
5. Remove cells in solution to a conical tube
6. Centrifuge at a low speed, ~2min until a cell pellet forms. Aspirate supernatant.
7. Add 5-10ml cell media. Mix gently by pipet.
8. Remove 10ul to a hemocytometer
    a. Clean the hemocytometer with ethanol and a kim wipe
9. Under a microscope, count all the cells in the 4 boxes surrounding the center grid. Calculate # of cells per ml media:
    a. total # cells counted / 4 = average # cells in 100nl
    b. average # cells in 100nl * 10,000 = average # cells in 1ml
    c. average # cells in 1ml * total ml in conical = total # cells
10. Centrifuge. Aspriate supernatant.
11. Resuspend cell pellet at 1ml freezing media per 1 million cells ($1x10^6$)
    a. Metabolism of DMSO in the freezing media will kill cells, therefore work quickly to get the cells to the freezer
12. Add 1ml cell suspension to each cryovial
13. Transfer the cryovials to a Mr. Frosty container, and place in a -80C freezer overnight
14. Transfer the cells from the -80C freezer to a liquid nitrogen freezer