Pullen Lab Protocol for Immortalized Adherent Cell Culture

This protocol is standard for immortalized mammalian sterile tissue culture. Varying cell lines may require slight changes to timelines or macronutrients supplied to the culture. All work done exposing cell cultures to open air should be done under the hood (biosafety cabinet).

1. Prepare complete RPMI 1640 (cRPMI) by the following amounts (v/v %):
    a. 1% 1M HEPES buffer
    b. 1% 10,000 units/mL Penicillin/10,000 µg/mL streptomycin
    c. 1% 200mM L-Glutamine
    d. 1% 100mM Sodium pyruvate
    e. 10% FB Essence (FBE), a serum cocktail
    f. 86% RPMI 1640
2. Warm cRPMI from previous step to 37^{o}C in water bath.
3. Remove selected cryovial from liquid nitrogen and immediately place in water bath at 37^{o}C.
4. Under hood, place 11mL of 37^{o}C cRPMI into T-75 sterile tissue culture flask.
5. Pipette the contents of thawed cryovial into the same T-75 flask, bringing the volume to ~12mL.
6. Label the flask with the cell line, the passage number, the date the culture was started, and the initials of the individual growing the flask.
7. Incubate culture in incubator at 37^{o}C, ~5.0% CO_2 overnight.
8. The next day examine viability of cells at 100x magnification via phase contrast microscopy.
9. If there is a large amount of attachment and growth, replace growth media with 12mL of fresh 37^{o}C cRPMI.
10. Following three or so days, the cell culture should be fed via replacing their growth media with 12mL of fresh 37^{o}C cRPMI.
11. In about a week, the cells will need to be passaged into a new container which is done via the following protocol laid out in steps 12-18.
12. Remove growth media from cell culture.
13. Pipette 3mL of trypsin onto culture to detach attached cells.
14. Place culture with trypsin into incubator for 3 minutes.
15. Immediately following step 13 place 6mL of 37^{o}C cRPMI. If this step is delayed too long, cells can be badly damaged.
16. Centrifuge resulting turbid solution at 1,400rpm to isolate cells from media.
17. Remove media and resuspend pellet in ~5mL of 37^{o}C cRPMI.
18. Place ~500µL of previous cell mixture into 12mL of cRPMI in T-75 and increase the passage number by one while labeling the flask.