Cell Freezing For Storage

There are several major protocols to follow when freezing cells:

1. Stable cell lines (HEK, NHDFs etc.) using DMEM+FBS+DMSO
2. Pluripotent stem cells and early differentiation (KOSR + DMSO)
3. Differentiated cardiomyocytes (Bam banker-not working yet)

Material

- FBS
- KOSR
- DMSO
- Tryple
- PBS
- Filter 0.45 m
- Freezing Vials
- Freezing Chamber

Preparation

- Label freezing vials.
- Make sure freezing chamber is equlibrated to Room Temp
- Prepare freezing solution:
  - For stable cell lines(10% DMSO; 90% Culture medium with FBS; 1.5mL per vial)
  - For hPSCs and early derivated (10%DMSO; 90% KOSR)
- Filter freezing medium
- Put freezing medium on ice, use cold

Process

- Aspirate medium from the cells.
- Wash with PBS.
- Add tryple (2mL per 10cm dish).
- Incubate up to 5 min at 37 ◦ C and check for detachment of cells.
- Harvest cells in 10mL of media transfer to falcon tube.
- Centrifuge, discard pellet.
- Resuspend in 1mL of freezing medium and keep on ice.
- Count cells.
- Dilute to 1-2x10E6 cells/mL of freezing media.
- Pipette 1-1.5mL in the tubes (depends on the type).
- Put vials in the freezing chamber.
- Label the freezing chamber with name, hour, and date.
- Put in -80 ◦ C (>2h).
- Move to liquid nitrogen.