

## **Protocol for Cryopreserving and thawing adherent cells (i.e., MEFs, MSCs, etc.)**

### **I. Cryopreserving cells**

1. When cells are nearly confluent but still in logarithmic growth (~80-90% confluent) cells can be passaged or frozen down.
2. I usually make 4-5 vials of frozen cells per 150mm plate (I do not count cells). Begin by labeling the appropriate number of cryopreservation vials with all pertinent information, including type of cell (MSC, MEF, etc.), genotype (if necessary), passage number, date frozen down, your name, and notebook page to reference, if possible. Use waterproof/ ethanol-proof marker so that tubes stay labeled in liquid nitrogen.
3. Remove media from cells. Wash once with PBS. Adherent cells must be trypsinized- add a small volume of 1X trypsin solution to dish (I try to use as little as possible- 7-8 mL per 150mm dish- just enough to cover the bottom). Watch cells closely- will usually be ready to passage in 5-10 minutes. Cells will become more rounded and may detach from plate when ready.
4. Add media to trypsinized cells- I usually add 10-15mLs. Use pipet to dissociate cells- pipet up and down several times, aiming media stream at different areas of dish (you can often see by eye where cells are still attached to dish).
5. Transfer media and cells to conical tube. Centrifuge at 1000 RPM in tabletop centrifuge.
6. Aspirate media. Resuspend cell pellet in freezing media (regular cell specific media plus 10% DMSO) at a volume equal to 1mL freezing media per vial (eg., for 8 vials of frozen cells add 8mL media).
7. Once cells are thoroughly resuspended, carefully pipet 1mL into each vial.
8. Transfer vials to Styrofoam rack (I use the ones from 15mL conical tubes). Once vials are in place, cover vials with another Styrofoam rack and tape together. Place entire thing in -80°C overnight. This step is extremely important because cells **MUST** be frozen slowly- the Styrofoam permits this.
9. Vials can be transferred to the liquid nitrogen tank the following day.
10. Make sure to retain an accurate record of how many vials there are and which box they are in.

Solutions:

Freezing Media: 90% regular cell-specific media plus 10% DMSO

### **II. Thawing cells**

1. Remove vial from liquid nitrogen, placing immediately on ice.
2. In the hood, get all reagents and dishes ready to use. Put appropriate volume of prewarmed media into dish(es). I usually plate 1 vial of frozen cells onto 2-100mm dishes or 1-150mm dish.
3. Place cells directly from ice into 37°C water bath- it is important to freeze cells slowly and thaw them quickly.
4. As soon as cells are completely thawed (~1 minute or so), pipet up and down slowly with a p1000 to resuspend cells (they will often settle on the bottom of the vial) and transfer them dropwise to already prepared plate containing media. Mix gently to disperse cells and place in 37°C incubator overnight.
5. The next morning change media to get rid of residual DMSO and dead cells. Grow as usual.