SOP: **Slow thawing of cryopreserved cells**
Date modified: **03/13/2015**
Modified by: **J. Halow and R. S. Hansen (UW Stam Lab)**

Summary

The following protocol describes the slow thawing of cryopreserved cells from any source.

Materials for Slow Thawing Cryopreserved Hematopoietic Cells

1. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
2. Characterized Fetal Bovine Serum (FBS) (HyClone, Cat# SH30071)
3. 70% Ethanol
4. 0.22µm Corning Filter Systems (Cat# 431097 for 500mL and Cat# 431098 for 1L)
5. Corning conical centrifuge tubes (15mL and 50mL)
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Hemocytometer
8. Eppendorf Centrifuge 5810R
9. Thermolyne Locator 4 liquid nitrogen freezer
10. 37°C water bath

Cell Slow Thawing Procedure

1. Remove cells from liquid nitrogen storage and thaw rapidly in a 37°C water bath.
2. Swab outside surface of cryovial with 70% ethanol and transfer cells to 50mL conical centrifuge tube.
3. Dilute cells with cell thawing buffer (0.22µm filter-sterilized room temperature PBS supplemented with 1% FBS) by making four dilutions as follows (from a starting cell volume of 1mL):
    a. add 1mL, thawing buffer slowly, dropwise, mixing with slow, gentle swirling and let equilibrate for 3 min (2mL total volume).
    b. add 2mL, thawing buffer slowly, dropwise, mixing with slow, gentle swirling and let equilibrate for 3 min (4mL total volume).
    c. add 8mL, thawing buffer slowly, mixing with slow, gentle swirling and let equilibrate for 3 min (12mL total volume).
    d. add 20mL, thawing buffer slowly, mixing with slow, gentle swirling and let equilibrate for 3 min (32mL final total volume).
4. Centrifuge at 470 x g for 10 min at room temperature.
5. Carefully remove supernatant and disturb pellet by raking the tube bottom against a tube rack.
6. Wash once with thawing buffer as in steps 4 and 5, and resuspend in cold 1X PBS for further processing. Do a cell count with the hemocytometer and determine the amount of cells necessary for DNaseI treatment, crosslinking for ChIP, and/or RNA experimentation.