Thawing CHO Cells Jon Sack April 11, 2011 Ken Eum July 9, 2012

Basic Idea- Thaw fast in a happy place

- 1. Add 5ml of cell media (F12 + 10% FBS) without antibiotics or selection agent to two 25cm<sup>2</sup> flasks and place into 37° C incubator for 1 hour
- 2. Remove cells from liquid nitrogen freezer
  - make sure to transport on dry ice and note removal in the liquid nitrogen log
  - KEEP THE CELLS FROZEN ON DRY ICE
- 3. Place tube of cells into the water bath for 1-2 min
  - Thaw the cells quickly
- 4. As soon as the cell are thawed, add 2 drops to 1 flask and the rest to the  $2^{nd}$  flask
- 5. Place flasks into the 37° C incubator
- 6. Change media 2 hours later with pen/strep
  - No selection agents
- 7. Change media 4-20 hours later, include pen/strep and selection agents
- 8. Split cells before 80% confluent