Sansam Lab
April 2022

Protocol for Freezing Cells

1) Grow cells until full but not completely confluent
2) Harvest cells into 15mL conical tube
3) Spin cells at 1,000 RPM (173xg) for 5min.
4) Aspirate media and resuspend in 1mL freezing media per freezing vial
5) Transfer vials to -80C (use a Styrofoam rack if available)
6) For long-term storage transfer tubes from -80C into liquid nitrogen

10cm plate freeze into 1-2 vials
15cm plate freeze into 3-4 vials

Freezing Media
20mL FBS
25mL media
5mL DMSO
Filter sterilize using 50mL Steriflip vacuum filter unit

Thawing cells

1) Remove vial from -80C or liquid nitrogen and place in 37C water bath just long enough for thaw.
2) Immediately upon thaw, transfer thawed cells into a 15mL conical tube containing 5mL fresh cell culture media
3) Spin cells at 1,000 RPM (173xg) for 5min.
4) Aspirate cell culture media and resuspend in fresh cell culture media
5) Plate cells
6) Next day: remove media from plate and add fresh media or split cells if the plate is full