Mouse Sperm Cryopreservation

Mutant Mouse Resource and Research Center

University of Missouri

4011 Discovery Drive, Columbia, MO, 65201, USA

Sperm Cryoprotective Media Worksheet

Today's Date: _____ Water Lot Number: _____

Add ~80% of final volume to a Griffin beaker ☐

Add the following reagents to the Griffin beaker with embryo water.

Catalog #	Reagent	g/100mL	Actual Amount Added	Lot number
Fisher, 232100	Skim Milk, Dehydrated	4.50		

Centrifuge the solution for 1 hour at 12,000 rpm (18,500g), at 4°C ☐

Remove supernatant. Approximate amount obtained: _____

Add additional embryo water to bring solution to ~80% of final volume ☐

Add the following reagents to the solution:

Catalog #	Reagent	g/100mL	Actual Amount Added	Lot number
Sigma M6145	1-Thioglycerol	4 **µL**		
Sigma R-0514	Raffinose Pentahydrate	18		

- Bring to final volume ☐
- Osmolality (expected 420-450 mOsm): _____
- Filter with 0.2 µm sterile filter unit ☐
- Aliquot into 15 mL conical tubes and label each tube with "Sperm Freezing Media, Osmolality, date and initials" and place in -20°C freezer ☐

Expiration Date (12 months): _____ **Batch # (JuLian Date): _____**

Sperm Cryopreservation

Materials:

- VersiDry Lab Soaker for benchtop
- 35 mm Petri dishes (Falcon 1008)
- Kimwipes
- Nitrile Gloves
- Micro-scissors
- Fine forceps
- Curved, serrated forceps
- Stereo microscope
- Disposal bags
- Sperm cryoprotective medium (CPM) (1.5 mL needed per male)
- Dulbecco's PBS (Gibco, 14287080) or FHM (or comparable)
- 37°C warming plate
- 5 mL syringe (or similar size)
- Straw adapter attached to syringe (IMV, 017276)
- 0.5 mL microcentrifuge tubes
- Pipette tips
- Pre-labeled CBS embryo/sperm straws
- Weights for straws
- Heat sealer
- Liquid Nitrogen (LN2)
- Safety glasses
- Styrofoam box for sperm freezing
- Metal tube rack
- Timer

Setup:

1. For each male prepare six Cryo BioSystem 0.5 mL high security embryo and sperm straws with appropriate label and weight. (Prepare 5 straws if not performing post-thaw quality control (QC) analyses)
2. Place 1.5 mL CPM in a 35 mm petri dish; prepare one dish per male.
3. For each male having sperm cryopreserved, prepare a 0.5 mL tube with 90 microliters of dPBS or FHM and a microscope slide with cover slip. These will be used to assess pre-freeze motility, concentration, and morphology.
4. Prepare a Styrofoam sperm freezing box with LN_2 to the marked line (approximately 1.5-2 inches) with LN_2. Place the metal tube rack on top of 2 plastic cane sleeves in the box as shown in Figure 2.
5. Set up dissection area and acquire animals.

Procedure:

1. Euthanize one male mouse.
2. Dissect and remove both cauda epididymides and the vasa deferentia (Figure 1) from the male.
3. Remove any fat and tease out any blood from the cauda epididymides and the vasa deferentia.
4. Transfer the tissues to the 1.5 mL of CPM. Cut each cauda epididymis 3-6 times and squeeze out the sperm from each vas deferens.
5. After 10 minutes gently swirl the tissues to create a uniform solution, then remove and discard the tissue.
6. Collect a 10 µL sample and place in the 0.5 mL tube containing 90 microliters of PBS or FHM. Acquire a small sample using a transfer pipet by capillary action and place on the microscope slide and cover with the cover slip.
    a. These samples will be used for pre-freeze analysis of motility, concentration, and morphology.
7. Load one straw with approximately 50 µL of the sperm solution to use for post-thaw QC analysis.
8. Load the remaining 5 straws with approximately 0.3 mL of the sperm solution. Seal both ends of the straw with a straw sealer.
9. Place the straws on the metal tube rack in the sperm freezing box and put the lid on the box. (Figure 2)
10. Allow the straws to remain in the vapor for a minimum of 5 minutes but no more than 30 minutes.
11. Plunge the straws directly into the LN_2.
12. Straws can be moved to a LN_2 Dewar for long-term storage.
13. Repeat this procedure for all males being frozen.

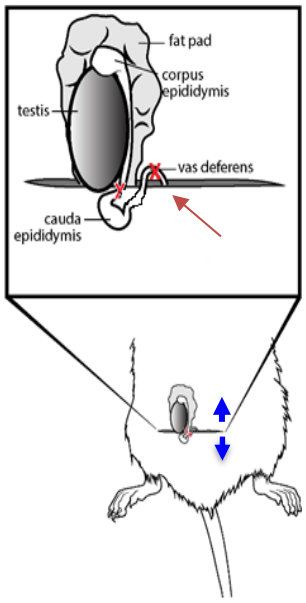

Figure 1. Dissection of reproductive organs of a male mouse. The position of the small lateral incision in the skin is indicated. The skin is then pulled back in the direction of the solid blue arrows. The body wall (peritoneum) is then cut to expose the testicle and accessary reproductive tracts (adapted from Behringer et al., 2014. Manipulating the mouse embryo (4rd edition), Cold Spring Harbor Laboratory Press, New York).

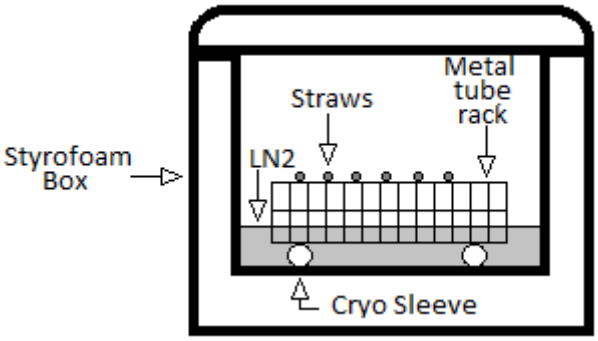

Figure 2. Sperm freezing box showing the layout of LN_2, metal tube rack and straws.

Thawing Sperm for QC Analysis or IVF

Materials:

- Nitrile Gloves
- Scissors
- 5 mL syringe (or similar size)
- Straw adapter attached to syringe (IMV, 017276)
- 1.5 mL microcentrifuge tubes (1 per sample)
- FHM media or comparable
- Centrifuge
- Hamilton Thorne IVOS or similar for QC analysis
- IVF dish containing TYH+MBCD media for IVF

Procedure:

1. Remove straw from liquid nitrogen and place in a 37°C **water bath** for 10 minutes.
    a. For QC analysis, use the straw containing the ~50 µL sample.
2. Place 1 mL of pre-warmed (37°C) FHM or comparable media into 1.5 mL microcentrifuge tube.
3. Remove straw from the water bath and dry the outside with a Kimwipe.
4. Cut the end of the straw opposite the weight and label, then insert into 1.5 mL tube and cut the other end.
5. Attach the syringe with straw adapter to the straw to gently expel remaining sperm.
6. Centrifuge the tube for five minutes at 300 rcf.
7. Remove the supernatant to leave approximately 100 µL in the tube.
8. For QC analysis:
    a. Place the tube in a 37°C bead bath for 30 minutes to allow sperm to swim out.
    b. Analyze the sample using Hamilton Thorne IVOS or comparable machine.
9. For IVF:
    a. Gently remove sperm and pellet from tube using a 200 µL pipette.
    b. Slowly add to an IVF dish containing TYH+MBCD media and place in 37°C incubator for 30 minutes prior to using for IVF (see IVF protocol for details).