Protocol for Cryodropper Cryopreservation of Mouse Sperm

Sperm Freezing

1. Prepare 2 male mice (>8 weeks old with proven fertility) by mating (plug positive) 4-7 days prior to sperm cryopreservation.
2. Prepare 1 sperm dish per mouse by depositing a 60µl drop of CARD CPA on a 35mm dish. Cover the drop with paraffin oil. Add a second 60µl aliquot of CPA to the first drop to make a tall, semi-spherical drop of CPA. Equilibrate at 37˚C (not in CO_2).
3. Label each Cryodropper. [We color code or label just the bulb portion.]
4. Prepare Cryodroppers: prepare 90µl drops of CARD PM medium in a dish, 1 drop per Cryodropper. Let equilibrate at 37˚C and 5% CO_2 for 10 min. Load drops into droppers by pipetting and gently flick the medium to the bulb portion. Gently squeeze the bulb to remove any medium remaining in the sperm loading area and wipe the liquid off with a Kimwipe. Store open end up in an Eppendorf rack at 37˚C with 5% CO_2 until sample loading.
5. Set up the freezing box with LN_2 and allow to cool.
6. Label and precool the cryogenic storage vials. [We use 4ml cryovials and label as needed. 4 Cryodroppers will fit per vial.]
7. Euthanize the mice.
8. Remove the cauda epididymides. Place them on a kimwipe and, under a microscope, completely remove all fat and blood.
9. Transfer one epididymis from each male to each sperm dish. This keeps the sperm from two males mixed in the sperm dishes. Note: the following steps must be performed quickly; under 30 minutes total from sperm release to freezing.
10. Using watchmaker's forceps and small angles scissors, make at least 6 incisions in each epididymis.
11. Place dish on slide warmer at 37˚C for 3 minutes. Rotate dish every minute to disperse sperm from tissue. Gently squeeze remaining sperm from the tissue as the tissue is removed from the medium.
12. Loading Cryodroppers: Using a gel loading pipette, carefully load the straw portion with 10µl sperm suspension in the center. Seal with a heat sealer. Put loaded prototypes directly on float in LN_2 vapor for 10 minutes.
13. Transfer Cryodroppers to vials. The vials are then transferred to a LN_2 dewar and stored in the vapor phase.

Sperm Thaw

1. Remove cryodropper from LN_2 storage.
2. Immerse in a 37˚C water bath until thawed and incubate for 10 minutes.
3. Remove device from water bath and gently dry with a Kimwipe.
4. Using scissors, cut the tip of the Cryodropper off and transfer sperm drop to a fresh IVF dish.
5. Gently shake CARD PM medium to end of device with single flick of wrist. [Too much force and the medium will be lost.]
6. Apply medium to sperm drop. [Note: If an IVF dish is used, the humidity should be maintained by water in the outer chamber. If a regular petri dish is used, the sperm drop should be covered with Paraffin equilibrated in the CO_2 incubator for at least 30 minutes prior to sperm thaw.]
7. Incubate in the CO_2 incubator at 37˚C to capacitate. [We generally capacitate ~45 minutes.]

References:

Adapted by B. Stone from Behringer R, Gertsenstein M, Vintersten K, Nagy A. 2014. Manipulating the mouse embryo: a laboratory manual, 4thed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

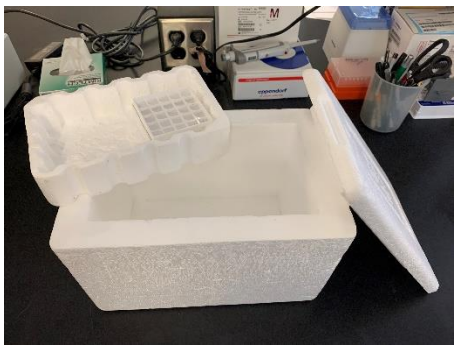
Cryodropper Video
https://youtu.be/J3iSHdoJi2E

Media Required:

Paraffin Oil, (Sigma-Aldrich 18512)
FERTIUP cryoprotectant (CPA) (Cosmobio KYD-001) (Can be made in-house as well, Behringer et al., pg. 674-675)
FERTIUP preincubation medium (PM) (Cosmobio KYD-002) (Can be made in-house as well, Behringer et al., pg. 614)

Equipment:

Cryodropper for Sperm Vitrification (red line: ParaTechs 80020)
Cryovial (4 ml) or other suitable LN_2 storage option (USA Scientific 1440-9100)
CO_2 incubator at 37˚C
Slide warmer or 37˚C incubator
Water bath @ 37˚C (500ml beaker of water in a 37˚C incubator works well)
Gel loading tips (ex: USA Scientific 1022-0600)
Pipettors and tips; ex: 1ml, 200ul, 20ul, 2ul
Eppendorf rack
Portable LN_2 vapor phase freezer with Styrofoam raft (see photo)
Sharpie for labeling
Kimwipes
Tissue culture dishes; 35mm, IVF dishes (optional)
Stereomicroscope
Timer
Impulse heat sealer (American International Electric AIE-105T)
Forceps (Watchmakers #5)
Scissors, dissection and small angled
LN_2 vapor phase storage dewar



Animals:

Male mice for sperm collection (proven fertility, mated 4-7 days prior to collection)