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<b>Protocol Section:</b>	<b>Cryopreservation</b>	<b>Policy No:</b>	P-CMMR4-SPC-00
<b>Protocol Subject:</b>	<b>Sperm Cryopreservation For ENU &amp; Non-ENU Mice</b>	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	08 March 2004
		<b>Date Revised:</b>	

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### Protocol Statement:

Much of the basic research in mammalian genetics and early development is undertaken with mice. Sperm cryopreservation procedures require a careful consideration of the complexity of the sperm plasma membrane, the interaction of its components and the influence of cooling, freezing and thawing on these interactions. Mouse sperm is extremely sensitive to the freezing and thawing process, when handling the sperm some precautions are necessary to minimize stress which would decrease the sperm quality:

1. Sperm collection must be performed gently (pipetting, expelling and transferring);
2. To prevent cold shock effects, cryopreservation medium (CPA) should be pre-warmed at 37 °C in an incubator before adding it to sperm suspension;
3. During the freezing and thawing stage, cryovials should be handled gently but quickly to prevent cold, osmotic and mechanic shock effects.

### 1. Apparatus

- 1.1 Fine tip forceps
- 1.2 Iris scissors
- 1.3 35mm petri dish
- 1.4 200µl wide bore pipette tips
- 1.5 1ml cryovials
- 1.6 37°C, 5% CO<sub>2</sub> incubator
- 1.7 Sigma R-763 D(+) Raffinose Pentahydrate
- 1.8 Difco 232100 Skim Milk Dehydrated

### 2.0. Collection of mouse sperm

- 2.1 Remove both epididymus and vas deferens aseptically from the male mouse at room temperature.
- 2.2 Transfer the epididymus and vas deferens to a 35mm petri dish containing 1mL of filtered CPA pre-stored in the incubator at 37°C, 5% CO<sub>2</sub>.
- 2.3 "Walk out" by carefully gliding to expel the sperms off half the vas deferens with a set of sterile fine-tipped forceps. Cut the vas deferens from the epididymus and pierce the epididymus about 5-7 time using the forceps..
- 2.4 Allow the sperm to "swim out" from the tissues into the cyroprotectant for 10 minutes in the incubator at 37°C, 5% CO<sub>2</sub>.
- 2.5 Remove debris and tissue with a pair of sterile blunt forceps and gently swirl the dish of sperm-cyroprotectant sample.

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### 3.0. Freezing of sperm

- 3.1 Transfer 8 x 100µl aliquots of the sperm/CPA suspension to 1ml cryovials using the wide bore pipette tips. Place cryovials into a rack which sits above the liquid nitrogen by 2.5cm. The cryovials will be exposed to the liquid nitrogen vapours (approximately -120°C) for 10 minutes at a cooling rate of 20-40°C per minute.
- 3.2 After 10 minutes plunge the cryovials directly into liquid nitrogen for storage.

### 4.0. Thawing of frozen sperm

- 4.1 Remove the cryovials of frozen sperm from liquid nitrogen storage loosening the lids to allow pressure release. Quickly and place the vials into a waterbath at 35-37°C until all crystals are melted (approximately 2 minutes). Caution: if liquid nitrogen seeped into the vial, open the lid and pour out the liquid nitrogen before thawing in waterbath.
- 4.2 Sperm is ready to be used in IVF.

**Issued by Lab Manager:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by Facility Management:** \_\_\_\_\_ **Date:** \_\_\_\_\_