JAX®
SPERM CRYOPRESERVATION KIT

Instructions for Preserving Your Mouse Strain

The Jackson Laboratory

Leading the search for tomorrow's cures

JAX® Sperm Cryopreservation Kit
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Critical Elements
JAX® Sperm Cryo Kit

In 2006, scientists at The Jackson Laboratory developed an innovative and reliable method of cryopreserving mouse sperm.1 To date, thousands of unique strains have been successfully cryopreserved and recovered using this method. The JAX® Sperm Cryopreservation Kit enables researchers to employ these new methods in their own laboratory.

Critical Elements to Consider for Using This Kit

This kit contains sufficient supplies and reagents to conduct one practice run, prior to cryopreservation of sperm from your mice. Successful use of the kit depends on several elements which are outlined below.

Do not dispose of the styrofoam box or styrofoam insert (raft). The styrofoam components are part of the freezing apparatus.

Upon arrival, store cryoprotective medium (CPM) at -80°C. CPM is perishable and must be used within the expiration date. Once a vial is thawed, the CPM should be stored at 4°C and used within seven days.

Successful sperm cryopreservation depends on accurate dissection of the vas deferens and cauda epididymis. Individuals are expected to have or to obtain experience in these dissection skills prior to using this kit. The Appendix provides a summary of vas deferens and cauda epididymis dissection but is not intended to replace requisite training.

For each strain, the sperm from two males must be collected, loaded into the straws and cassettes, and placed into liquid nitrogen vapor (LN2) within 30 minutes. Prior to cryopreserving your strains, use the practice supplies to familiarize yourself with the protocol and appropriate timing.

A Model Information Form and pre-labeled straws and cassettes are provided for each strain. Complete the Model Information Form prior to cryopreserving sperm. Be sure the unique ID strain code on the Model Information Form matches the straws and cassettes for each strain and corresponds to the particular strain being cryopreserved.

Removal of live colonies of cryopreserved strains from the shelf is NOT RECOMMENDED until satisfactory quality control results are obtained.

[1]Ostermeier GC, Wiles MV, Farley JS, Taft RA. 2008. Conserving, Distributing and Managing Genetically Modified Mouse Lines by Sperm Cryopreservation. *PLoS ONE* 3(7): e2792 doi:10.1371/journal.pone.0002792

Kit Contents
JAX® Sperm Cryo Kit

JAX® Sperm Cryopreservation Kit Contents

Item	Purpose	Storage
Styrofoam freezing box and lid with 3.6 cm thick walls (27.5 cm x 22.6 cm x 27.7 cm)	Freeze sperm	
Styrofoam raft (polystyrene insulation board,18.3 cm x 13.4 cm x 3.6 cm)	Platform to float samples in liquid nitrogen vapor	
Cryoprotective medium (CPM)	Collect and freeze sperm	Note expiration date and store at -80°C. Once thawed, store at 4°C for no more than 7 days
35 mm tissue culture dishes	Collect sperm	
0.25 ml French straws	Cryopreservation vessel	
Monoject syringes (1 cc TB regular Luer tip)	Load straws	
Heat sealer or Critoseal™ straw sealing powder	Seal straws	Keep dry at room temperature
Cassettes and straws	Cassettes contain the straws during freezing and storage	
Model Information Form	Link straws and cassettes to specific strains and investigators	

Required User Supplies and Skills

Item/Skill	Purpose
Dissection expertise	Successful sperm cryopreservation depends on accurate dissection of the vas deferens and cauda epididymis (Appendix)
Two healthy male mice for each strain (ideally 10-16 weeks of age) housed alone for one week	Provide sperm
Liquid Nitrogen (LN_2)	Freeze sperm
Standard personal protective equipment for handling LN_2, including LN_2 gloves, forceps and eye protection, and as required by your facility.	Protect user
Timer	Time sperm incubation, straw loading and exposure of sperm to LN_2 vapor
37°C slide warmer or non-CO_2 incubator	Bring CPM in tissue culture dishes to appropriate temperature and maintain that temperature
70% Ethanol (EtOH) in spray bottle	Wet and cleanse mouse abdomen and clean dissection tools
Sharp tip 5 and 3 inch scissors or other fine dissection scissors Two serrated micro dissecting forceps Two Dumont forceps or other fine dissection forceps 26 gauge needles and 1 ml syringe	Dissect cauda epididymis and vas deferens
1 ml pipettor and tips	Aliquot CPM
500 ml beaker filled with two inches of room temperature tap water	Seal straws
Large forceps (25 cm)	Tip styrofoam raft and retrieve cassettes in LN_2
37°C water bath	Thaw CPM
10X dissecting microscope with transmitted light base	View and dissect tissues

Protocol for Using the JAX® Sperm Cryo Kit

Step 1. Thaw the cryoprotective medium

For each strain to be cryopreserved, thaw one 3 ml tube of cryoprotective media (CPM) by placing it in a 37°C water bath for approximately 30 minutes. There may be a precipitate at the bottom of the tube. Oscillate well and continue to incubate at 37°C until all of the precipitate is dissolved.

NOTE: Please utilize the media prior to the expiration date.

NOTE: Once CPM is thawed, do not refreeze. It can be stored at 4°C for up to seven days if necessary.

Be sure to warm CPM to 37°C and oscillate to dissolve precipitate prior to use.

Step 2. Complete the Model Information Form

Complete the Model Information Form, providing strain information and the unique Strain ID (Fig. 1) printed on the straws and cassettes that will be used for each strain.

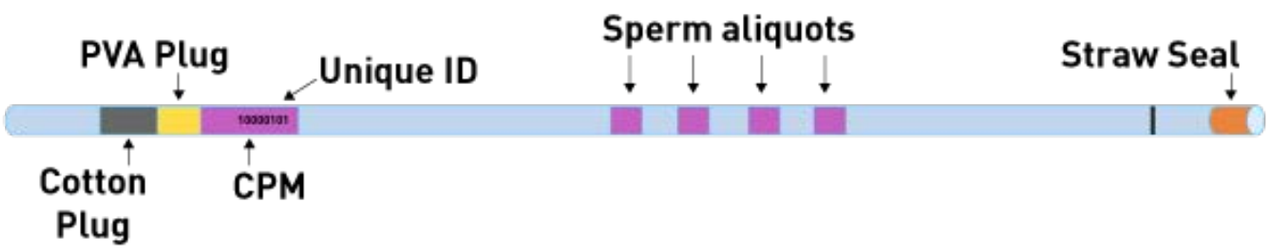


Figure 1. *Straw labels. Each straw is labeled with a unique Strain ID for tracking strain information.*

Step 3. Prepare styrofoam freezing apparatus (Fig. 2)

NOTE: Follow standard lab safety practices when handling LN_2.

a. Remove the styrofoam freezing box from the cardboard shipping box and remove all contents

b. Fill the freezing box with LN_2 up to the Liquid Nitrogen Fill Line. If you can't see the Liquid Nitrogen Fill Line on the box through the LN_2 vapor, place the lid on the box and wait one to two minutes for the vapor to dissipate.

c. Place the styrofoam raft in freezing box, such that it floats on top of the LN_2 and replace the lid.

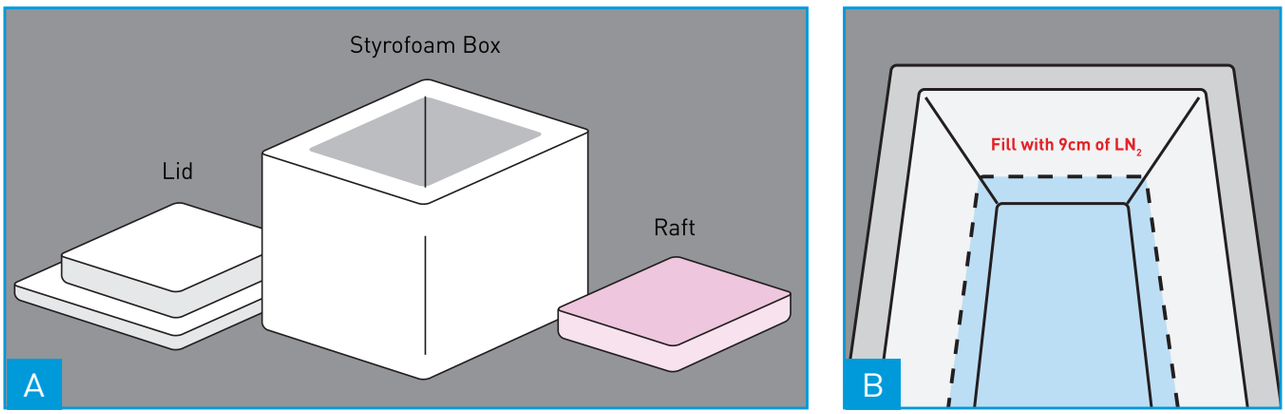


Figure 2. Sperm freezing apparatus. A. The sperm freezing apparatus includes the styrofoam box, lid and raft B. The freezing box filled with LN_2 up to the Liquid Nitrogen Fill Line.

Step 4. Prepare collection dish

a. Label a 35 mm tissue culture dish (provided) using the Strain ID number, pipette 1 ml of CPM per male into the dish, and replace the lid. This will be the sperm solution dish. Place on a 37°C slide warmer or into non- CO_2 incubator at 37°C.

NOTE: Do not incubate with CO_2 as this may change the pH of the CPM and adversely affect cryopreservation success. This dish will be used to collect four vas deferentia/cauda epididymides from two males of the same strain.

b. Pipette remaining CPM into a second tissue culture dish labeled "CPM Only", replace lid and store at room temperature.

Step 5. Collect Sperm

NOTE: For each strain, the sperm from two males must be collected, loaded into the straws and cassettes, and placed into liquid nitrogen vapor (LN_2) within 30 minutes.

a. For the first strain to be cryopreserved, euthanize two healthy males of the same strain (ideally 10-16 weeks of age and housed alone for one week) following your institutional Animal Care and Use Committee protocol.

b. Immediately collect the vas deferentia/cauda epididymides from each male (Appendix). Place four vas deferentia/cauda epididymides into the sperm solution dish labeled with the corresponding ID (**Fig. 3a**).

c. View vas deferentia/ cauda epididymides in CPM using a 10X dissecting microscope with transmitted light base. Secure the cauda epididymides one at a time with a clean Dumont forceps and make seven to eight cuts in each using a new 26 gauge needle or a pair of fine dissection scissors **(Fig. 3b-c)**. Sperm will be visible as a slowly diffusing, cloudy mass when viewed through the dissecting microscope.

d. Gently swirl the dish to ensure sperm has moved to medium. Allow the sperm to swim out of the tissue for 10 minutes at 37°C (non-CO2) **(Fig. 3d)**. Clean dissection instruments by wiping with 70% EtOH.

e. Remove and discard the tissues from the collection dish and again gently swirl the collection dish to distribute sperm. Leave the sperm solution at room temperature and immediately begin loading the straws.

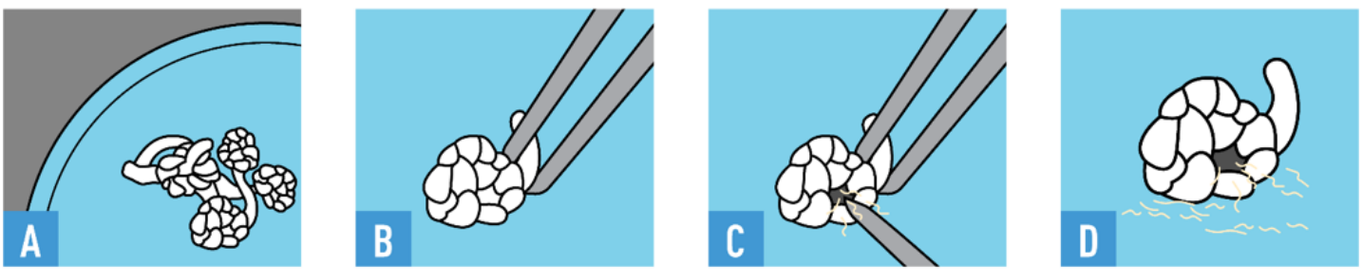


Figure 3. Sperm Collection. A. Vasa deferentia and cauda epididymides in CPM. B-C. Epididymis secured with forceps and cut with a 26 gauge needle. D. Diffusing sperm.

Step 6. Fill straws and load cassettes

NOTE: Use no more than 10 minutes to fill straws and place cassettes on the raft in LN_2 vapor.

a. Attach a monoject syringe to the cotton/PVA plug end of a straw (**Fig. 4a**).

NOTE: Be sure the unique Strain ID numbers on the straws being filled are the same as the ID number listed on the Model Information Form for that particular strain.

b. Aspirate CPM from the "CPM Only" dish to the first mark on the straw **(Fig. 4b-I)**. This creates the ballast. Remove the straw from the CPM and aspirate air until the **top** of the CPM ballast reaches the second mark on the straw. (**Fig. 4b-II**).

c. Aspirate the sperm solution (from the tissue culture dish labeled with the ID) until the top of the ballast reaches third mark on the straw. This creates the first 10 µl (0.4 cm) sperm aliquot **(Fig. 4b-III)**. Aspirate air until the ballast reaches the fourth mark on the straw. **(Fig. 4b-IV)**.

d. Repeat step 6c alternating aspiration of sperm solution and air, until you have four individual 10 µl (0.4 cm) sperm solution aliquots separated by air pockets **(Fig. 4b-V through IX)**.

IMPORTANT NOTE: Handle loaded cassettes with care. Avoid bumping or dropping them as this can cause plastic to shatter and membranes to rupture.

e. Aspirate air until the CPM ballast wets the cotton/PVA plug **(Fig. 4b-X)**. Remove the straw from the syringe. Handle the straws gently. If a straw is bumped or shaken and the sperm aliquots combine, discard the straw and fill a new one.

f. Seal each straw by gently tapping the open end of the straw vertically into the straw sealing powder until a plug of about 0.25 cm is formed **(Fig. 4c)**. Do not create too large a sealing plug, as this may lead to powder mixing with the sperm sample. Place powder-plugged end of straw into a 500 ml beaker filled with two inches of room temperature tap water for a minimum of 30 seconds. Wipe excess PVA from the outside of the straw. Accumulate straws in the beaker of water until all straws for a single strain, with the same ID number, are loaded.

g. Completely dry straws by gently rolling on a paper towel and place five straws into each cassette (with the same Strain ID numbers), straw seal first **(Fig. 4d)**. Slide the cassette plunger containing the straws into the clear sleeve until the cassette is completely closed.

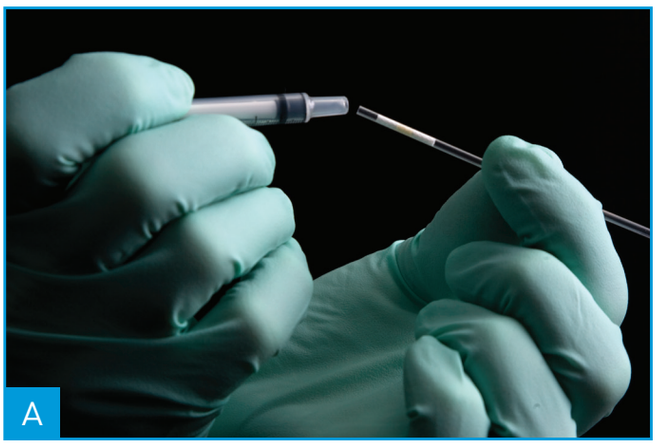


Figure 4. Filling straws.

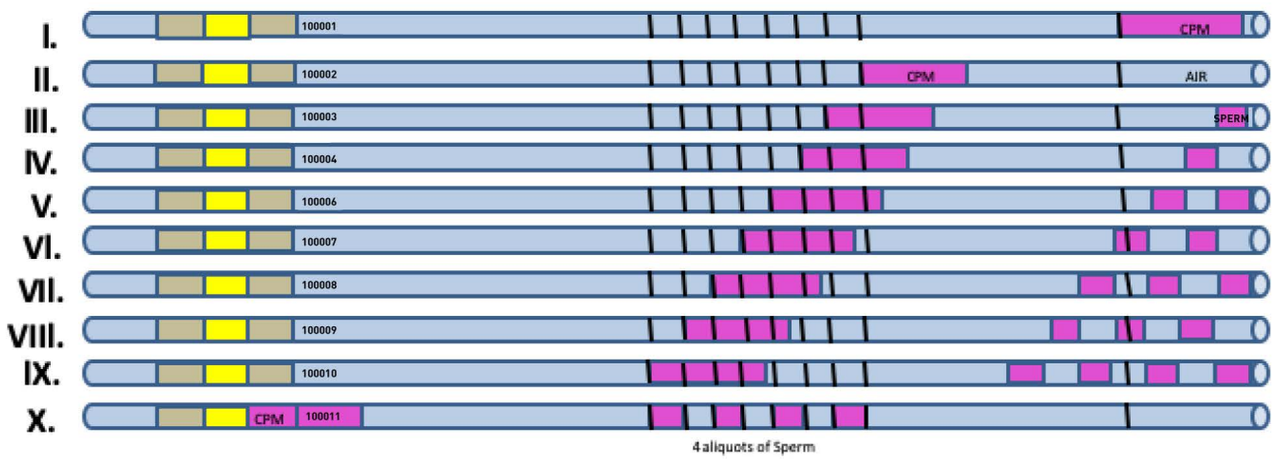
A. Monoject syringe being attached to the cotton/PVA plug end of a French straw.

B. Diagram of straw loading steps I – IX showing aspirated CPM ballast, air and sperm samples relative to the marks on the straw used to designate volumes.

C. Straw loaded with four sperm aliquots (separated by air pockets) and sealed with straw sealing powder.

D. Straws being loaded into a cassette, straw seal end first.

B



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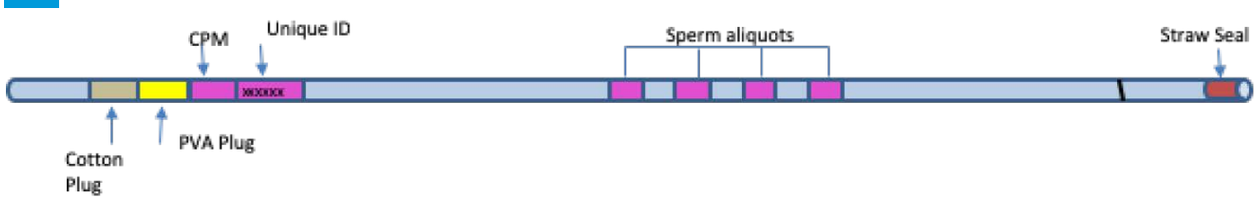
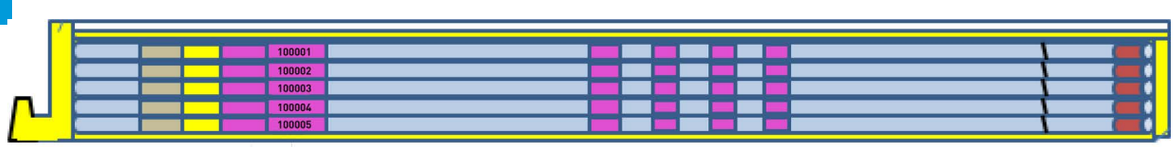


D



Step 7. Freeze samples

NOTE: Follow standard lab safety practices when handling LN_2

a. Once both cassettes are loaded (one strain with 10 straws), position the cassettes on the raft in LN_2 vapor of the sperm freezing box (**Fig. 5**) and replace the box lid.

NOTE: Handle loaded cassettes with care. Avoid bumping or dropping them as this can cause plastic to shatter and membranes to rupture.

b. Expose the samples to the LN_2 vapor for at least 10 minutes, but not longer than 30 minutes. Do not remove the lid or jostle the box during this time.

IMPORTANT NOTE: Do not allow cassettes to fall into LN_2 prior to completing the 10 minute exposure to LN_2 vapor. If this occurs, these samples should be discarded.

c. Upon completing the 10-30 minute vapor cooling phase, use long forceps to tip the raft so the cassettes are plunged directly into the LN_2.

d. Repeat procedure until all strains are cryopreserved, monitoring the LN_2 level in the freezing box at the start of each new strain.

IMPORTANT NOTE: Use a new tissue culture dish and a new 26 gauge needle for each strain, and clean dissection tools with 70% EtOH between strains to prevent cross contamination of sperm samples.

e. Store cryopreserved in liquid nitrogen.

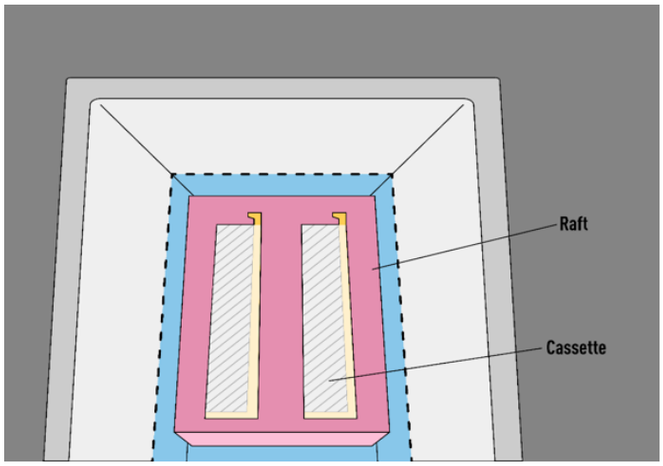


Figure 5. Freezing samples.
Cassettes placed flat on top of the raft to cool in LN_2 vapor.

Contact Information

For questions regarding the use of this kit, contact
Technical Information Services at The Jackson Laboratory:

Monday - Friday 8:00am - 8:00pm (ET)
Toll free: 1-800-422-6423
International: +1-207-288-5845
Web: jax.org/technical-information

jax.org/sperm-cryo-kit

Product License Agreement

JAX® Sperm Cryo Kit

PRODUCT LICENSE AGREEMENT FOR JAX® MOUSE SPERM CRYOPRESERVATION KIT: **READ THIS BEFORE OPENING THE KIT AND BEFORE USING.**

Thank you for selecting the Mouse Sperm Cryopreservation Kit ("Kit") from The Jackson Laboratory ("Jackson"). The Kit is provided to you under the terms of this no-fee-use license. **BY OPENING THE KIT YOU ARE INDICATING ACCEPTANCE OF THESE TERMS.**

If you do not accept these terms, return the Kit and proof of purchase for a complete refund.

Use of the Kit

By purchasing the Kit, Jackson grants you a limited right to use Jackson intellectual property incorporated in the Kit as follows:

1. You may use the Kit for the cryopreservation of mouse sperm as described in the Manual enclosed with the Kit.

2. You may not re-sell the Kit.

3. You may not use the Kit to provide services to other parties without the prior written consent of Jackson.

4. You may not use the Kit with human derived materials, whether in clinical trials, therapeutic, preventative, diagnostic or any other purposes.

THIS LICENSE WILL TERMINATE AUTOMATICALLY if you fail to comply with the terms and conditions set forth above.

Limited Warranty

EXCEPT AS SPECIFIED IN THIS AGREEMENT, THERE ARE NO WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, OR ANY WARRANTY OF NON-INFRINGEMENT OF ANY INTELLECTUAL PROPERTY RIGHTS.

The cryoprotective medium is perishable. The shelf life of the medium at -80°C is at least twelve (12) months (refer to the labeled expiration on the individual container); therefore, the Kit must be used within this timeframe. Once the container is opened, the cryoprotective medium should be stored at 4°C and used within seven (7) days. The limited warranty is void for incorrectly stored cryoprotective medium or cryoprotective medium that is older than twelve (12) months.

If you find your Kit to be defective on delivery, you shall notify us in writing immediately and at Jackson's choice it will be replaced or credited ("Limited Warranty").

THESE ARE YOUR SOLE AND EXCLUSIVE REMEDIES for any and all claims that you may have against Jackson arising out of or in connection with this product, whether made or suffered by you or another person and whether based in contract or tort.

IN NO EVENT WILL JACKSON BE LIABLE TO YOU OR ANY OTHER PARTY FOR DIRECT, INDIRECT, GENERAL, SPECIAL, INCIDENTAL, CONSEQUENTIAL, EXEMPLARY OR OTHER DAMAGES ARISING FROM THE USE OF OR INABILITY TO USE THE KIT OR FROM ANY BREACH OF THIS WARRANTY, EVEN IF JACKSON HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

You assume full responsibility for the selection of the Kit, and for the use and the results of that use.

Other Provisions

This agreement constitutes the entire agreement between you and Jackson and supercedes any prior understandings and agreements, either oral or written with respect to the Kit. This no-fee-use license agreement shall be interpreted under the laws of the State of Maine, without reference to conflict of laws priniciples.

No action for breach of the Limited Warranty may be commenced more than one (1) year following the expiration date of the above Limited Warranty.

Jackson strongly recommends that you maintain any live colonies of strains that have been cryopreserved until you have received and approved the results of quality control from Jackson. See additional services for Sperm Cryo Kit for details.

Appendix: Dissection of vas deferentia and cauda epididymides

IMPORTANT NOTE: Successful sperm cryopreservation depends on accurate dissection of the vas deferens and cauda epididymis. Individuals are expected to have, or to obtain, experience in this dissection skill prior to using this kit. This appendix provides a summary of the dissection of the vas deferens and cauda epididymis but is not intended to replace requisite training. To ensure the highest sperm quality, the total dissection time for two males should be less than five minutes.

1. Euthanize two healthy males (ideally 10-16 weeks of age) of the same strain, following your institutional Animal Care and Use Committee protocol. Place both euthanized males on absorbent paper and generously spray abdomens with 70% EtOH **(Fig. 1a)**. Using a serrated micro dissecting forcep, lift a small fold of skin in the center of the abdomen level with the top of the legs and make a small horizontal cut with five inch scissors. Pull the skin in opposite directions (towards the head and tail) until the peritoneum is completely exposed **(Fig. 1b)**.
2. Using the second serrated micro dissecting forcep, lift a small fold of the peritoneum and make a small cut using fine dissection scissors to allow air to enter the abdominal cavity. Cut the peritoneum to expose the abdominal cavity, using caution not to cut any of the coils of the gut or any of the internal organs (**Fig. 1c)**. Locate the testicular fat pads and urinary bladder **(Fig. 1d).** If the urinary bladder is not visible, push the testicular fat pads away from the midline.
3. Using the urinary bladder as a landmark, locate both vas deferentia **(Fig. 1e)**. If they are not readily visible, gently grasp the cranial end of the urinary bladder with dissecting forceps. Staying on the midline, elevate the bladder upwards and caudally to expose the vas deferentia as they enter the urethra. Using forceps, grasp a vas deferens close to the urinary bladder. Cut the vas deferens, but not the blood vessel that runs along it. Pull the vas deferens away from the body leaving behind the blood vessel and connective tissue **(Fig. 1e)**.

   NOTE: Handle the vas deferens gently; it is delicate and can easily tear when pulled.

4. Follow the vas deferens until the cauda epididymis is found. Pull the vas deferens gently to expose the cauda epididymis completely. While pulling, cut the attached fat and ligament to release the cauda epididymis and vas deferens together (Fig. 1e). Leave behind fat, blood vessels, connective tissue, caput epididymis and testis. Place the tissues on a paper towel and collect the other vas deferens and cauda epididymis. Examine the tissues and blot them on the paper towel to remove any blood. Repeat for second male. Place the vas deferens and cauda epididymis from both males into the 2 ml CPM aliquot labeled with the ID number. You will have four vas deferentia/cauda epididymides from the same strain in the same tissue culture dish.

Appendix
JAX® Sperm Cryo Kit

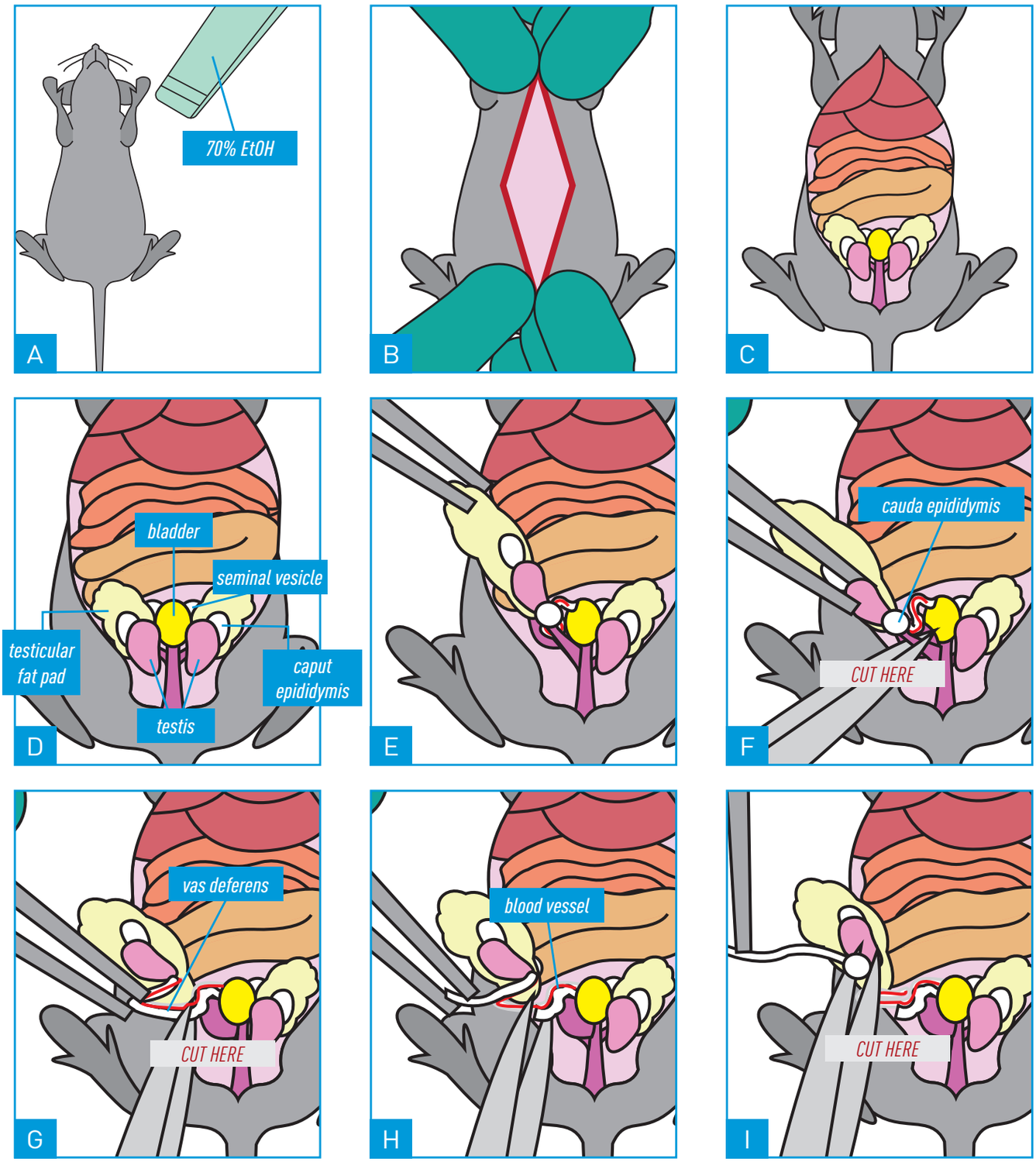


Figure 1. ***A.*** *Abdomen with 70% EtOH.* ***B.*** *Skin being pulled in opposite directions to expose peritoneum.* ***C-D.*** *Open body cavity exposing the testicular fat pads.* ***E.*** *Pull on a testicular fat pad to expose the attached testical.* ***F.*** *Locate the cauda epididymis and vas deferens. Cut the ligament adjacent to the cauda epididymis.* ***G.*** *Cut the vas deferens near the bladder.* ***H.*** *Gently pull the vas deferens away from the body cavity leaving behind connective tissue and blood vessel.* ***I.*** *Cut just below the cauda epididymis to liberate the tissue.*

The Jackson Laboratory

Leading the search for tomorrow's cures

Founded in 1929, The Jackson Laboratory is a nonprofit biomedical research institution dedicated to leading the search for tomorrow's cures.

Our mission: We discover precise genomic solutions for disease and empower the global biomedical community in our shared quest to improve human health.

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