REQUEST FOR CRYOPRESERVATION

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Please check cryopreservation service(s) requested and complete the appropriate sections:

____ A. Cryopreservation of mouse embryos

____ B. Cryopreservation of mouse sperm

____ C. Generation of mice from cryopreserved embryos by embryo transfers

____ D. Generation of mice from cryopreserved sperm by IVF and embryo transfers

Principal investigator (PI) _____

PI phone # _____

PI FAX # _____

PI route # _____

PI Email address _____

Account # for charges _____

A. Cryopreservation of mouse embryos

Service: For each day of cryopreservation requested, we will isolate and cryopreserve 8-cell embryos from as many as 15 superovulated female mice using the slow cooling method. We will also isolate and cryopreserve 8-cell embryos from 5 superovulated mice as controls for viability. These control embryos will be thawed and cultured *in vitro* to determine their viability (as measured by the number of 8-cell embryos advancing to the blastocyst stage). The cost for this service is $450[1] per day of cryopreservation. The investigator is responsible for supplying the male mice needed for mating and the superovulated females (from both the strain and control mice). If we need to purchase mice, we will charge the investigator for the cost of purchasing these mice, the shipping charges, and the cost of housing the mice prior to superovulation.

General Information

1. The number of 8-cell embryos obtained from superovulation varies greatly from mouse to mouse (The average from C57BL/6 is 10-20 per female), and is dependent upon the strain, the genetic background, and the age of the mouse. For many strains (i.e. C57BL/6), optimal superovulation is obtained with 4-6 week old females. Some superovulated

females may not have any 8-cell embryos, and some females may not mate successfully with the males.

2. We recommend cryopreserving at least 250 embryos, but more if affordable. We recommend combining sperm cryopreservation with embryo cryopreservation.

3. If mice are housed in the MRB barrier, we will perform the superovulation and check for copulatory plugs. If mice are housed in a different facility, we will provide instructions and/or a demonstration how to superovulate the females and check for copulatory plugs.

4. We will provide cryovials containing 30-40 embryos to the investigator. These cryovials should be stored in the liquid phase of liquid nitrogen, preferably in multiple locations.

5. Our viability for cryopreserved control embryos has been 60%. We predict that ~25% of viable thawed embryos will be recovered as liveborn mice (i.e., from 100 cryopreserved embryos- 15-liveborn mice). The number of liveborn mice recovered from frozen embryos can vary widely from strain to strain. Embryos from transgenic or knockout mice may have inherently reduced viability.

6. We cannot guarantee the ease of recovery of cryopreserved embryos, since it can vary from strain-to-strain. We can thaw cryopreserved embryos and transfer these embryos into foster mothers to determine the ease of recovery of a strain. We recommend this service prior to discontinuing maintenance of a valuable stock. Please complete section C to request this service.

Name of strain _____

Genetic Background _____

Age of males _____ Coat color _____

Age of females _____ Coat color _____

Generation of males (i.e. F1, N9, etc.) _____

Generation of females _____

Is strain being maintained as: Heterozygotes____ Homozygotes ____

Where are mice currently located? _____

How many embryos would you like to cryopreserve? _____
We will estimate the number of cryopreservation sessions that will be required prior to initiating the request.

Please verify that you are not providing the facility your only stocks of your strain (in case the yield of embryos is low).

_____ Yes, I have other mice that can be used to propagate this strain

B. Cryopreservation of mouse sperm

Service: We will cryopreserve sperm from a male mouse and distribute sperm into 8 cryovials. We will perform a sperm count and a sperm motility count of unfrozen sperm. The cost for this service is $50[1] per male mouse. If requested, we will thaw one vial and perform *in vitro* fertilization of eggs from 5 superovulated mice to determine the ability of the sperm to fertilize mouse eggs. We will also perform a sperm count and a sperm motility count on thawed sperm. We recommend this service prior to discontinuing maintenance of a valuable stock. Please complete section D of this form if you are interested in this service.

General Information
1. The optimal age of the males is 2-3 months. Sperm from inbred mice vary dramatically in their ability to fertilize mouse eggs. Sperm from F1 hybrids are more efficient for *in vitro* fertilization. We strongly recommend freezing sperm from F1 hybrid rather than inbred mice. Historically, sperm from a C57BL/6 genetic background have been very difficult to recover by *in vitro* fertilization.

2. We recommend cryopreserving sperm from 5 F1 hybrid mice from each strain.
We also recommend combining sperm cryopreservation with embryo cryopreservation.

3. These cryovials should be stored in the liquid phase of liquid nitrogen, preferably in multiple locations.

Name of strain _____

Genetic Background _____

Age of males _____

# of mice _____ Generation_____

Expected Coat color _____
(of offspring)

Describe how the mouse line has been maintained (i.e. as backcrosses, intercrosses).

Is strain being maintained as: Heterozygotes____ Homozygotes ____

Where are mice currently housed? _____

Please verify that you are not providing the facility your only stocks of the strain (in the unlikely event of a technical problem).

_____ Yes, I have other mice that can be used to propagate this strain

C. Generation of mice from cryopreserved embryos by embryo transfers

Service: We will perform embryo transfers using cryopreserved embryos to generate mice. Please complete the form and we will provide a quote for the cost of this service. The per diem costs (currently $0.69/cage/day) will be the responsibility of the investigator, beginning with the day of the embryo transfers.

Name of strain _____

Expected coat color _____

vials to thaw _____

of embryos to transfer _____

Animal protocol # _____

Approved Animal Protocol Title

_____

_____

Most recent date of ACUC approval_____

Check stress level of your protocol (when they are transferred from our ACUC protocol to your ACUC protocol):

A____ B____ C____ D____ E____

D. Generation of mice from cryopreserved sperm by IVF and embryo transfers
Service: We will superovulate female mice to obtain unfertilized eggs. We will then perform *in vitro* fertilization with cryopreserved sperm and culture the eggs *in vitro* to determine whether they advance to the 2-cell stage. Finally, we will perform embryo transfers on 2-cell fertilized eggs. Please complete the form and we will provide a quote for the cost of this service. The per diem costs (currently $0.69/cage/day) will be the responsibility of the investigator, beginning with the day of the embryo transfers.

Name of strain _____

Expected coat color (of offspring) _____

Genotype(s) of cryopreserved sperm _____

Mouse strain for obtaining unfertilized eggs _____
(i.e. desired genetic background)

How many mice would you like to obtain? _____

Animal protocol # _____

Approved Animal Protocol Title

_____

_____

Most recent date of ACUC approval _____

Check stress level of your protocol (when they are transferred from our ACUC protocol to your ACUC protocol):

A____ B____ C____ D____ E____

[1]Fees are subject to change without notice (You will be informed at the time of the service request if fees have changed).

Return this form to Maki Wakamiya, Medical Research Bld. (Rt. 1048), Rm. 9.104, FAX: 409-747-1938, or Email mawakami@utmb.edu. If you have any questions, contact Maki Wakamiya (409-772-2811), Charlie Luo (747-2365) (zheluo@utmb.edu), or San Yang (747-2224) (sfyang@utmb.edu).

If you are requesting embryo transfers (services C and D), please sign the ARC animal transfer request form located on the next page (investigator signature in transfer to section). We will type in the remainder of the information.

Animal Transfer Request (PI to PI or Protocol to Protocol)

Today's Date _____ **Effective Date** _____

TRANSFER FROM:

P.I _____ **Signature:** _____

IACUC# _____ **Phone** _____

Account# _____ **P.O.#** _____

These animals have ____ **or have not** ____ **been used on the above listed protocol.**

TRANSFER To:

P.I _____ **Signature:** _____

IACUC _____ **Phone** _____

Account# _____ **(to be billed) Stress Level ….. A** ____ **B** ____ **C** ____ **D** ____ **E** ____

Species _____ **Strain** _____ **Sex** _____

Date of receipt _____ **Animal I.D.#** _____ **(large animals)**

Current Housing Location : _____ **Room#** _____

# of Animals to be Transferred _____ **# of Cages to be Transferred** _____

All portions of the form must be completed. Incomplete forms will be returned.

revised 12/03/98