REQUEST FOR KNOCKOUT MOUSE PRODUCTION

REQUEST# ___________ (to be filled in by facility)

DATE RECEIVED ______________________________ (to be filled in by facility)

Principal investigator (PI) ______________________________

PI phone # ______________________________

PI FAX # ______________________________

PI route # ______________________________

PI Email address ______________________________

Contact name & phone # ______________________________
(Person responsible for analyses of tail biopsies)

Name of construct ______________________________

Gene name ______________________________

Gene symbol ______________________________

Account # for production fee ______________________________

Account # for animal housing costs ______________________________

Building/Rm. # where tail biopsies should be brought

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Check services requested:

____ A. Identification of knockout embryonic stem (ES) cell lines

____ B. Microinjection of ES cells into mouse blastocysts

SERVICES PROVIDED:
A. Isolation of ES cell lines: We will perform one electroporation and pick cell lines that have survived selection (generally 300-500 per electroporation). We will cryopreserve each cell line and provide cellular extracts from each cell line for the isolation of DNA by the investigator. The investigator will analyze these DNAs by Southern blot

hybridization/ PCR to identify cell lines that have integrated the construct by homologous recombination. We will then prepare these cell lines for microinjection into mouse blastocysts (i.e., amplification of the cell line). We cannot guarantee that we will generate an ES cell line that has undergone homologous recombination (since the frequency of such an event could be low, with a range generally between 1:10 to 1:500).

B. Microinjection of ES cell lines into mouse blastocysts: We will microinject embryonic stem cell lines into mouse blastocysts and transfer the embryos into foster mothers. We will perform 4 days of microinjection for each week of microinjection requested by the investigator. Generation of a sufficient number of chimeric mice will require at least 2-3 weeks of injection. We will mate the chimeric mice and provide the investigator tail biopsies of their offspring so that the investigator can identify mice that have transmitted the knockout allele through the germline. We will then intercross heterozygous knockout mice and maintain these lines until the first litter is weaned. Finally, we will conduct an orientation to the investigator's lab (if needed) about how to perform tail biopsies and how to maintain the knockout lines.

COST SHARING: (1) Identification of knockout ES cell lines: $5400/electroporation[1]. (2) Microinjection of ES cells into mouse blastocysts: $4200/week of injection + $850 set up fee for the purchase of C57BL/6 male mice[1]. **The Sealy Center for Cancer Cell Biology and several departments have provided funds to subsidize production costs.** The cost of knockout mouse generation can vary from construct-to-construct and will depend upon: (1) whether more than one electroporation is required to generate the knockout embryonic stem cell lines and (2) the number of weeks of microinjection of the embryonic stem cell lines requested by the investigator. **In addition to the production fee, the investigator will also be charged for the housing of mice once the microinjected blastocysts have been transferred into recipient females. The current cost of animal housing is $0.69/cage/day.** This cost can be minimized if tail samples provided to the investigator are promptly analyzed to determine which mice carry the knockout allele.

A WORK REQUEST WILL NOT BEGIN UNTIL THE INVESTIGATOR HAS RECEIVED APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE AND BIOLOGICAL SAFETY COMMITTEE.

1. The generation of knockout mouse models often requires a significant amount of interaction between the investigator and the Director. Do you consider this project a collaboration? yes ___ no ___

2. A brief description of your project (include expected phenotype).

3. Has this construct received Biological Safety Committee approval? yes ___ no ___

4. Approved Animal Protocol Title

____

____

Animal protocol # _____________________________________

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____

5. Do you currently have space in an animal facility? yes ___ no ___

If you have space, where do you wish to have your animals housed after they are transferred from the Transgenic Mouse Core Facility?

If you do not have space, have you contacted the ARC to obtain space? yes ___ no ___

6. Attach a restriction endonuclease map of the targeting construct. Label all known restriction sites.

7. If you will be analyzing your DNAs by Southern blot hybridization, list one or more restriction endonucleases that you will use to detect the knockout allele. If you will be analyzing your DNAs by PCR, have you tested your primers to verify they amplify the expected fragment size(s)?

8. If you are requesting generation of ES cell lines, please provide the facility 300 μg of linearized DNA (cut 1X with a restriction enzyme at a site that flanks the insert; provide a picture of the gel). We may need additional DNA if more than one electroporation is required. A procedure for purification of DNA for electroporation is located at the end of this request.

9. You will be provided tail biopsies from which you will isolate DNAs for Southern blot analyses/PCR in order to identify mice that carry the knockout allele. Other services now offered by the facility are (1) isolation of genomic DNAs from tail biopsies of liveborn mice, and (2) analyses of these DNAs by Southern blot analyses. We charge $28.40/hour[1] for labor + the costs of materials for these services. For tail DNA isolation, the cost of materials is ~$0.20/tail[1]. For Southern blot analyses, the cost of materials will vary depending on the number of tails and the restriction endonuclease(s) needed for the analyses. Please indicate if you are interested in any of these services. We will let you know whether we will be able to provide these services within a reasonable amount of time (Our first priority is to generate transgenic/knockout mice). If your lab intends to genotype the mice, please indicate whether you will require assistance.

Require assistance with genotyping tail DNAs: Yes ____

Interested in the facility isolating tail DNAs and/or genotyping DNAs: Yes ____
Please complete the appropriate section(s) of the Special Services Request Form.

10. Following microinjection of ES cells into blastocytes and generation of chimeric mice, we will mate the chimeric male mice to two C57BL/6 female mice (unless you request us to mate one male with one female), in order to transmit the knockout allele through the germline. **We will charge $17.00[1] for each female mouse that we need for performing the matings.**

11. **The knockout mouse production fee includes setting up the matings for the heterozygous knockout mice and maintenance of the offspring until they are 20 days of age. At this time, the mice will be transferred to the care of the PI. We will provide an orientation (if needed) to your laboratory about how to collect tail biopsies and how to maintain knockout lines. If the investigator chooses, the Transgenic Mouse Core Facility can maintain knockout mouse stocks for a charge of $28.40/hr[1]. We will provide this service on a case-by-case basis. Preference will be given to the youngest knockout mouse strains and to collaborative projects. As space and technician time become limiting, it may be necessary to transfer the care of older knockout mouse stocks to the PI.**

12. Please sign the animal transfer form located on the next page. This form will authorize transfers for animals from our account to your account. This transaction will occur whenever microinjected blastocysts are transferred into foster mothers. We will send you a copy of each transaction. **We will be transferring animal care costs to your animal account, beginning with the day that we transfer microinjected blastocysts into foster mothers. Please be aware that the number of cages that you will be charged will increase when we separate pregnant females, wean mice, or set up matings. The ARC will bill you at the end of each month (and tell you the # of**

cages you have). Your cage charges can be minimized by prompt analyses of tail biopsies.

13. Please sign to verify that you understand the terms of this agreement.

PI_______________________________________

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

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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 (Ph. 409-772-2811), Charlie Luo (747-2365) (zheluo@utmb.edu),
or San Yang (747-2365) (sfyang@utmb.edu).

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

Preparation of DNA Construct for Electroporation

1. Digest 200 µg of DNA with a restriction endonuclease that cuts 1X in the plasmid at a site that flanks the insert. Preferably, this site should not be on the same side of the insert as PGK-TK.

2. Test a small aliquot for complete digestion on an analytical gel. We will do the remaining steps if you wish.

3. Extract the DNA 2X with phenol, 2X with phenol/chloroform and 2X with chloroform.

4. To supernatant, add 1/10 volume of 3M sodium acetate and 2.5 volumes of ethanol. The DNA should be clearly visible. Spool the DNA with a glass rod and transfer it to a tube containing 500 µl of 70% ethanol.

5. Wash DNA 2-3 times with 70% ethanol.

6. Resuspend pellet in TE at a concentration of ~5 µg/ul.

7. Determine the DNA concentration by measuring the OD reading. Store DNA at -20°C in 100-200 µg aliquots. Avoid repeated freeze/thaw cycles.