UAB TRANSGENIC ANIMAL/EMBRYONIC STEM CELL RESOURCE

INSTRUCTIONS TO USERS – ANIMAL COMPONENT

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Please note: Sections 3, 4 and 7 along with an internal requisition and accompanying documentation are required at the time of project submission. The services offered by the TA/ESC Resource are performed for research purposes only -- due to a number of patent-related restrictions (all of the processes used to generate genetically-engineered mice are patented by other entities). Please contact the UAB Research Foundation, should you have commercially-related concerns or considerations.

Contact
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Email: rozmahel@uab.edu

Effective: November 01, 2002
1. OUTLINE OF SERVICES, PRIORITIES, RESPONSIBILITIES AND PRODUCTIVITY

The services of the TA/ESC Resource are available to UAB investigators on a first-come first-serve basis. The Associate Director determines scheduling of all work; however, if assignment of project priorities becomes necessary, input from the Director and/or Advisory Committee would be solicited. Generally, priority will be given to extramurally funded projects.

An investigator may submit any number of projects annually. At this time, priority among applicants for services shall be established according to the following criteria: date of application, status as part of a funded research project, number of transgenic animals previously created by the Resource for the investigator, relevance of the transgenic model to funded research aims at UAB, as well as other criteria as shall be identified and set by the Resource Advisory Committee. The Advisory Committee for the TA/ESC Resource is composed of representative faculty users and key administrators. Members of the Advisory Committee include: D. Bullard (Advisory Committee Chairman), L.W. Gerrity, L. Guay-Woodford, J.F. Kearney, R.P. Kimberly, R. Rozmahel (Director, TA/ESC Resource), C. Weaver and P.A. Wood.

SERVICES

1. **DNA Microinjection**: A minimum of 150 inbred C57BL/6 or B6xSJL F2 fertilized oocytes will be microinjected with the DNA construct (transgene), per experiment. For transgenes that are not lethal, in excess of 12 founder animals are expected to result per experiment.

2. **ES Cell Transfer**: Twenty C57BL/6 blastocysts will be injected with an Investigator-provided ES cell clone. Depending on the quality of the ES cells, a minimum of 4 chimeric mice should result.

3. **Re-Derivation of Mouse Lines**: By *in vitro* fertilization of oocytes from 12 superovulated B6xSJL F1 females with sperm from Investigator-provided male mice (line to be re-derived) and transfer to a maximum of 4 recipient females. Alternatives for re-derivation including C-section and fostering, use of Investigator-provided breeding pairs (for homozygous offspring), different mouse strains, etc., can be accommodated by the TA/ESC at an additional charge to recover added mouse acquisition and *per diem* costs.

4. **Embryo Cryopreservation**: By *in vitro* fertilization of oocytes from 12 superovulated B6xSJL F1 females using sperm from Investigator-provided males (line to be cryopreserved). Fertilized oocytes are stored for up to one year. Embryo recovery and transfer to a maximum of 3 recipients within one-year is included in this service. Modifications of the service such as utilization of Investigator-provided breeding pairs (for homozygous embryos), alternate mouse strains, etc., can also be accommodated by the TA/ESC at an additional charge to recover mouse costs and *per diem* charges.

5. **Other Services**: In addition to the primary embryo experiments, the TA/ESC Resource can provide or assist Investigators with other services relating to mouse embryo studies, mouse development or line maintenance. Other services include timed embryo collection, assisted reproductive techniques (i.e., *in vitro* fertilization [IVF], superovulation, and embryo transfer), etc. Please contact the Resource regarding the cost of these other services. The TA/ESC Resource also provides complete mouse ES cell and gene targeting services and support. Please refer to the **ES Cell Component Schedule** for further details regarding mouse ES cell and gene-targeting services.
**RESOURCE RESPONSIBILITIES**

**DNA Microinjection:** The TA/ESC Resource will microinject 150 fertilized C57BL/6 or B6JxSJL F2 hybrid oocytes with your DNA fragment and re-implant them into pseudopregnant recipient females. For transgenes that are not lethal, in excess of 12 founder animals are expected to result per experiment. If less than 12 animals are delivered, the TA/ESC Resource will re-inject the transgene construct into an additional 75 oocytes, this will be the extent of the guarantee. In our experience, these numbers are sufficient to generate 4-8 founder transgenic mice. The Investigator will be informed of the dates of injection to prepare for the mouse shipments and identification of transgenic founders in their own lab. The 2-week old pups and their mothers (recipient females) will be transferred to the Investigator 5 weeks after the microinjection experiment (prior to weaning). Although the TA/ESC Resource guarantees to generate a minimum of 12 founder animals per experiment, a “best effort” assurance only applies. If alternative mouse strains are requested, there will be no assurance with the exception of a “best effort” only guarantee for a one day only set up - if fertilized oocyte yield is below 150, costs will be incurred as indicated in Section 2, and the project considered completed.

**ES Cell Injection:** The TA/ESC Resource will inject mouse ES cells into 20 3.5-day old C57BL/6 blastocysts, followed by their re-implantation into the appropriate number of pseudopregnant recipient females. These procedures will be performed on a "per day" basis with the expectation that a minimum of 20 blastocysts will be injected. This number should be sufficient to generate more than 4 founder chimeric animals; however, the success of these experiments depends largely on the quality (pluripotency) of the Investigator-provided ES cell line. There will be a “best effort” guarantee only. If alternative mouse strains are requested, there will be no assurance with the exception of a “best effort” only guarantee for a one day only set up - if blastocyst yield is below 20, costs will be incurred as indicated in Section 2, and the project considered completed.

**Re-Derivation and Cryopreservation of Mouse Lines:** The TA/ESC Resource cannot accommodate projects requiring a containment level greater than NIH RAC BL2. Although every reasonable effort will be made for the successful outcome of the experiment, there will be a “best effort” guarantee only.

All husbandry practices for Resource animals are those of strict barrier maintenance. Microisolator cages are used, changed only in laminar flow change stations by trained personnel wearing protective garb. Cages, food, bedding, water bottles, etc., are sterilized.

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**INVESTIGATOR RESPONSIBILITIES**

**DNA Microinjection:** It is the responsibility of the Investigator to provide to the TA/ESC Resource an appropriately prepared DNA sample at the required concentration (please see Section 5 for DNA preparation and concentration information) for oocyte microinjection at time of project submission. If necessary, exceptions to the time of submission rule are possible; however, the Associate Director must authorize such exceptions at time of project submission. The Investigator is solely responsible for the quality of the sample; a $100 non-refundable fee will be assessed if the submitted DNA sample is found to be unsuitable for microinjection. In the event that the Investigator chooses to postpone or cancel the experiment after scheduling, a one week advanced notice communicated directly to the Associate Director is required. The Investigator will receive the resultant mice and
their transfer recipient mothers at 2 weeks of age (1 week before weaning) for confirmation and analysis of the transgene.

**ES Cell Microinjection**: It is the responsibility of the *Investigator* to provide to the TA/ESC Resource a clean (no bacterial or fungal contamination, nor presence of antibacterial or antifungal drugs) ES cell line plated at 50% confluency on the morning that the experiment is scheduled. Please refer to Section 6 for detailed information related to ES Cell Preparation Guidelines. If the ES cells submitted for blastocyst microinjections are found to be unsuitable (insufficient number of cells or contamination), the experiment will not proceed and the *Investigator* shall forfeit the service charge. In the event that the *Investigator* chooses to postpone or cancel the experiment after scheduling, a two week advanced notice communicated directly to the Associate Director is required. The *Investigator* will receive the resultant mice and their transfer recipient mothers at 2 weeks of age (1 week before weaning) for confirmation and analysis of germline transmission of the genetic modification.

**Re-Derivation and Cryopreservation of Mouse Lines**: It is the responsibility of the *Investigator* to arrange for these experiments with the Associate Director of the TA/ESC Resource a minimum of 3 weeks before the animals are transferred to the Resource. It is also the responsibility of the *Investigator* to ensure that the animals provided for this service are of the correct genotype and strain, and appropriate arrangements are made with respect to their housing and containment. Only animals from approved sources, known to be free of pathogens (based on UAB health surveillance test results) are allowed entry into the Resource. The TA/ESC Resource cannot accommodate projects requiring a containment level greater than NIH RAC BL2.

**PRODUCTIVITY**

The Resource is fully operational and mice are generated using either DNA microinjection or ES cell transfer techniques. To date, the UAB TA/ESC Resource has generated in excess of 5000 genetically modified founder mice containing transgenes or gene-targeted ES cells, and more than 60 UAB investigators have received expert assistance from, or consulted with, the Resource in their research efforts.
2. SERVICE COSTS

Primary Services:

<table>
<thead>
<tr>
<th>Project Type</th>
<th>Mouse Strain</th>
<th>Guarantee</th>
<th>TOTAL COST</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Microinjection¹</td>
<td>B6xSJL F2 hybrid</td>
<td>150 fertilized oocytes injected, 12 live mice delivered*</td>
<td>$500</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td></td>
<td>$750</td>
</tr>
<tr>
<td></td>
<td>Other Strains</td>
<td></td>
<td>$850³</td>
</tr>
<tr>
<td>ES Cell Transfer²</td>
<td>C57BL/6</td>
<td>Inject ≥20 blastocysts</td>
<td>$500</td>
</tr>
<tr>
<td></td>
<td>Other Strains</td>
<td></td>
<td>$850³</td>
</tr>
<tr>
<td>Line Rederivation</td>
<td>B6xSJL F1 hybrid</td>
<td>None</td>
<td>$500</td>
</tr>
<tr>
<td>Embryo Cryopreservation</td>
<td>B6xSJL F1 hybrid</td>
<td>None</td>
<td>$500</td>
</tr>
</tbody>
</table>

Note: On average, 1/4 of the mice delivered should be transgenic. We anticipate approximately 20-25 mice per experiment with an average of 4-8 transgenic mice. Transgenic mice are not guaranteed—this service provides a "best effort" assurance only, see Section 1. ¹A $100 non-refundable fee will be assessed if the DNA sample submitted is found to be unsuitable for microinjection. ²If the ES cells submitted for blastocyst microinjections are found to be unsuitable (insufficient number of cells or contamination), the experiment will not proceed and the Investigator will forfeit the service charge. ³Plus additional mouse purchase and per diem recovery costs.

Additional Mouse Costs:

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Cost</th>
<th>Minimum Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (B6xSJL F1 hybrid or C57BL/6)</td>
<td>$8 per mouse</td>
<td>$25 per order</td>
</tr>
<tr>
<td>Female B6xSJL F1 hybrid</td>
<td>$6 per mouse</td>
<td>$25 per order</td>
</tr>
<tr>
<td>Female C57BL/6</td>
<td>$8 per mouse</td>
<td>$25 per order</td>
</tr>
<tr>
<td>Lactating females (±pups)</td>
<td>$25 each</td>
<td>$25 per order</td>
</tr>
</tbody>
</table>
3. PROJECT SUBMISSION FORM - TA/ESC RESOURCE – ANIMAL COMPONENT

DATE: ___________________________________________

NAME: ___________________________________________

DEPARTMENT: _______________________________________

ADDRESS: _________________________________________

PHONE/FAX: _______________________________________

E-MAIL: ___________________________________________

UAB ACCOUNT: _____________________________________

EXTRAMURAL SUPPORT ID/GRANT #: __________________

UAB CENTER MEMBERSHIP (CIRCLE): CCC MAMDC OTHER: _________________

IACUC PROJECT APPROVAL #: _________________________

WHERE ARE ANIMALS TO BE HOUSED?: ______________________________

PROJECT NAME (12 character limit): __________________________

STRAIN/SPECIAL REQUIREMENTS: _____________________________

DNA Microinjection: (DNA concentration/volume/buffer/size (kb)):________________________

ES Cell Transfer: (mycoplasma test result, date): ________________________________

**BRIEF PROJECT DESCRIPTION:**

PLEAASE ATTACH: 1) gel photo of final aliquot for DNA microinjection, 2) restriction map (including fragment length and restriction enzyme cut sites), and 3) Sections 4 and 7, and an internal requisition for anticipated costs.

Date Received: ___________________________ By: ___________________________
4. UAB MOUSE TRANSFER INFORMATION

[This page will be forwarded to the UAB ARP]

REQUEST FOR ANIMAL PROCUREMENT

University of Alabama at Birmingham
Animal Resources Program
Volker Hall B10
Phone: 4-3408  Fax: 4-1188

DATE OF REQUEST: ________________________________

UAB ACCOUNT NUMBER: ____________________________

AUS APPROVAL NUMBER: ____________________________

STRESS LEVEL: ____________________________

INVESTIGATOR: ____________________________

DEPARTMENT: ____________________________

LAB CONTACT: ____________________________

VENDOR: UAB TA/ES Cell Resource

DATE NEEDED: When Available

<table>
<thead>
<tr>
<th>QUANTITY</th>
<th>SPECIES/DESCRIPTION</th>
<th>WEIGHT</th>
<th>SEX</th>
<th>AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Embryo transfer recipients with 2 week old litters</td>
<td></td>
<td>F</td>
<td>10-16 wks</td>
</tr>
</tbody>
</table>

SPECIAL REQUIREMENTS:

________________________________________________________________________________________

________________________________________________________________________________________

________________________________________________________________________________________

ARP Business Office Use Only Below This Line

Order Number:

Date Form Received in ARP: ____________________________

Received By: ____________________________

Date Order Placed: ____________________________

Placed By: ____________________________

To Be Housed: ____________________________

Date Received: ____________________________

Date Noted on Census: ____________________________

Date Billed: ____________________________

Vendor Invoice Number: ____________________________

Invoice Date: ____________________________
5. Guidelines for DNA Fragment (Transgene) Preparation for Oocyte Microinjection

The transgene construct should be cloned in an appropriate vector (e.g., plasmid or cosmid); the DNA purified (e.g., if a plasmid, by CsCl gradient or by ion exchange column chromatography [e.g., a Bethesda Research Laboratories NACS system] and digested with appropriate restriction enzyme(s) to remove all or most extraneous (cloning vector) sequences. The linearized transgene DNA fragment should then be isolated by gel electrophoresis and purified to the appropriate concentration before providing it to the TA/ESC Resource for microinjection. The fragment can be concentrated using a DNA extraction protocol such as glass bead purification (e.g., Qiagen Qiaex II), or electroelution, ion exchange chromatography, and ethanol precipitation (e.g., NACS or a Schleicher & Shuell Elutip protocols). These final steps should provide a purified sample completely free of particulate matter for oocyte microinjection.

The final transgene DNA sample can be submitted either as an ethanol precipitate or in solution in TE buffer (10 mM Tris, 0.25 mM EDTA, pH 7.5, DNA concentration in excess of 10 ng/μl). If the fragment is shipped in TE buffer it should be packed on ice. A minimum of 1 μg of purified fragment is required for oocyte microinjection. Specification of the sample buffer, exact concentration, volume and size of your final DNA sample is required to appropriately dilute it for microinjection.

*Upon receiving the DNA fragment (transgene construct) TA/ESC Resource staff will confirm its purity and concentration using agarose gel electrophoresis. If (i) multiple bands appear, (ii) contamination is evident, or (iii) there is less than 1 μg of total DNA, all remaining samples of the DNA construct shall be returned to Investigator with an explanation and documentation, and the TA/ESC Resource shall not be obligated to proceed further with the experiment and the non-refundable $100 fee (as described in Section 2) will be forfeited.
6. Guidelines for ES Cell Preparation for Blastocyst Microinjection

We require mycoplasma testing of all cell lines (results of analyses must accompany the submission), and advise checking all cell lines for proper chromosome number prior to submission. For information regarding mycoplasma testing, counting of ES cell chromosomes, or should other questions regarding methodology arise, please contact the TA/ESC Resource. If new ES cell lines are to be used, we highly recommend testing the native cells for pluripotency and germ line transmission by blastocyst injection before proceeding to transfections.

Maintain the ES cells stored (5 x 10^6 cells in 1 ml vials) at −80 °C in freezing solution (high glucose DMEM with β-mercaptoethanol, NEAA, glutamine, sodium pyruvate, LIF, 25% heat inactivated FCS or FBS, and 10% DMSO.

**Preparation of ES Cells for Microinjection:**

1. Thaw one vial of frozen cells (5 x 10^6 cells) onto a P100 plate with feeder cells (primary embryonic fibroblast), or in some cases 2X concentration of LIF, 2 days before the scheduled microinjection experiment. **Change media daily.**

2. On the morning of the scheduled microinjection experiment, treat the P100 plate with 2 ml of 0.04% trypsin for 5 minutes, pipette up and down several times to detach and disaggregate the cells, and stop the trypsinization with 10 ml ES cell media. Spin the cells down lightly and resuspend in 1 ml of injection media (ES cell media with 20% FCS and 20 mM HEPES).

3. Keep ES cells on wet ice at all times and have ready (along with an additional 25 ml of injection media) for microinjection at 9 AM on the day scheduled for the experiment. It is the sole responsibility of the Investigator to bring the cells to the TA/ESC Resource, or make prior arrangements for their pick up, on the morning of the experiment.

*If the ES cells submitted for blastocyst microinjections are found to be unsuitable (insufficient number of cells or contamination), they will be returned to the Investigator with an explanation and documentation. The TA/ESC Resource is not obligated to proceed further and the Service Charge (described in Section 2) will be forfeited.
TA/ESC RESOURCE TRANSGENIC MOUSE PRODUCTION SERVICE AGREEMENT

This Agreement is made between the UAB TA/ESC Resource (herein, "Resource") and __________________________ (herein, "PI") for the purpose of developing transgenic and gene targeted (herein, genetically modified) mice for PI.

I. The Project. The Resource will (circle a, b or c): a) microinject DNA into C57BL/6xSJL F2 mouse oocytes to generate transgenic mice using the PI's DNA construct, b) transfer the PI's ES cells into mouse blastocysts to create chimeric mice, or c) Other ___________________________.

A. Resource Responsibilities: We will use reasonable efforts to create the genetically modified founder mice through a) microinjection of DNA into C57BL/6xSJL F2 mouse oocytes using the DNA fragment supplied by PI, b) transfer of mouse ES cells supplied by PI into blastocysts, or c) as defined above.

B. PI Responsibilities: PI agrees to provide the DNA construct, cell lines, or mice, as well as all disclosures and approvals required under this Agreement and by all UAB regulatory requirements.

II. DNA Construct or Cell Line Disclosure, Institutional Approvals, Restricted Genetic Materials and Condition of Genetic Materials

A. PI will provide the Resource with a full written disclosure of the nature of the DNA construct or cell lines, including a restriction map, transfection integration characteristics, if known, and original published references, if available.

B. PI hereby represents and warrants that the DNA construct(s) or cell line(s) will not produce any infectious condition that may be harmful to other animals, humans or the environment, and that the experiments do not require containment conditions greater than NIH RAC BL-2 standards.

C. The Resource shall be entitled to 1) not commence its duties under Section I.A. until such time as it receives the written documentation required under Sections II.A. and II.B., and initial fees or requisitions under Section III.A., and 2) terminate this Agreement and the duties and responsibilities if found that (a) a DNA construct or cell line consists or contains, in whole or in part, a replication competent virus or recombinant DNA requiring containment in excess of NIH RAC BL-2 containment, or (b) for microinjection experiments finds, after examination by electrophoretic gel, that PI's DNA construct was not prepared according to the outlined guidelines or does not meet the stated requirements for microinjection (the specified fee [as identified on Page 5 of this document] would be forfeited).

III. Fee. Payment Schedule. Terms and Conditions

A. PI shall be billed for the total estimated charges upon delivery of each DNA construct or cell line to the Resource.

B. PI shall use the genetically modified mice produced under this contract solely for purposes of non-commercial research. PI shall not use the genetically modified mice other than as provided herein and shall not sell, lease, rent, barter away or otherwise transfer the genetically modified mice or any interest therein, except that the genetically modified mice may be transferred to a third party for purposes of breeding them solely for use by PI, except that genetically modified mice may be provided to other institutions for research purposes only. The PI shall notify the Resource of any such transfers of the genetically modified mice.

IV. Confidentiality

A. The Resource will hold in confidence the identity and nature of PI's projects and will limit disclosure of such matters to only Resource employees, provided however, that such confidentiality obligation does not apply to (i) information that is known to the Resource on the date hereof or becomes known to the Resource from a third party; or (ii) information that is required to be disclosed by applicable law or a governmental authority having jurisdiction.

B. Upon completion or termination of the Resource's duties and obligations, the Resource may retain a sample of the PI's materials and shall, if requested by PI, return any other remaining materials, proprietary information, cell lines or DNA constructs supplied by PI.

V. Miscellaneous

A. Upon PI's receipt of mice from the Resource, the PI agrees to assume full responsibility for such mice and all risks of harm they may cause including, but not limited to, any injury resulting from the handling of the mice.

B. PI agrees to assume responsibility for any claims of third parties based on or arising out of (i) a breach of PI's agreements, obligations, representations, or warranties made hereunder or pursuant hereto, (ii) any patent or other proprietary right infringement claim, which is brought with respect to the DNA construct or the Resource's use of said materials as contemplated herein or the use of any genetically modified mice by PI or by any third party who obtains such genetically modified mice from PI, or (iii) use, storage, handling, distribution, or disposal of any genetically modified mice by PI or by a third party who obtains such genetically modified mice from PI, to the extent provided under the Federal Tort Claims Act or other applicable Federal Statute.

C. PI hereby agrees that any publications involving the genetically modified mice provided hereunder shall acknowledge the "UAB Transgenic Animal Resource" as the source of the genetically modified mice. PI shall promptly provide copies of all such publications to the Resource.

D. PI hereby acknowledges that although the Resource will use all reasonable efforts to produce the genetically modified mice, it is not possible for the Resource to guarantee the successful production of the mice hereunder and that the Resource has made no representation or warranty herein to that effect.

E. This Agreement shall be construed, interpreted, and applied in accordance with the laws of (1) the State of Alabama and (2) regulations of the University of Alabama at Birmingham.

IN WITNESS WHEREOF, the duly authorized representatives of the parties have executed this Agreement to be effective on _____________.

THE RESOURCE                                                                                     THE PI

By:                                                                                              By:_______________________________________
Name:                                                                                             Name:_______________________________________
Date:                                                                                              Date:_______________________________________