

# UAB Transgenic Mouse Facility



## INSTRUCTIONS TO USERS & SERVICE AGREEMENT

SECTION	PAGE
Table of Contents .....	p. 1
1. Outline of Services, Priorities, Responsibilities and Productivity .....	p. 2-3
2. Service Fees .....	p. 4
3. Project Submission Form ( <b>required at time of project submission</b> ).....	p. 5-6
4. Guidelines for DNA Fragment Preparation for Pronuclear Microinjection	p. 7
5. Guidelines for ES Cell Preparation for Blastocyst Microinjection.....	p. 7
6. Service Agreement ( <b>required at time of project submission</b> ).....	p. 8

**Please note that the following documents are required at the time of project submission:**

- **Section 3 (Project Submission Form)**
- **Section 6 (Contract, signed by the *Investigator*)**
- **Internal Payment Form (with Oracle account number)**

The services offered by the UAB Transgenic Mouse Facility (TMF) are performed for research purposes only to non-profit and academic institutions 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.

**Director:**

**Bob Kesterson, PhD**  
Kaul Human Genetics Bld Rm 602A  
Telephone: (205) 934-7206  
[bkesterson@genetics.uab.edu](mailto:bkesterson@genetics.uab.edu)

**Coordinator and Information Contact:**

**Larry W. Johnson, M.S.**  
Kaul Human Genetics Bld 606  
Telephone: (205) 934-2998  
[lwj@uab.edu](mailto:lwj@uab.edu)

## **1. OUTLINE OF SERVICES, PRIORITIES, RESPONSIBILITIES AND PRODUCTIVITY**

The TMF provides state of the art services to assist UAB investigators in their studies to create transgenic mouse models. These services are available to UAB investigators on a first-come, first-served basis. The Coordinator determines scheduling of all work with consultation from the Director.

### **SERVICES**

In order to facilitate a positive interaction between investigators and the TMF staff, the following description of services and expectations has been established. Please read through this description carefully and contact either the Coordinator or the Director for any clarifications.

- **DNA Microinjection:** A minimum of 150 inbred C57BL/6 (B6) or B6xSJL F2 fertilized oocytes will be microinjected with the DNA construct (transgene) per experiment. For transgenes that are not lethal, a minimum of 12 potential founder animals are expected to result per experiment.
- **ES Cell Transfer:** Forty or more C57BL/6 blastocysts will be injected with an *Investigator*-provided ES cell clone.
- **Gene Targeting:** Users are required to schedule a consultation meeting with the Director to discuss all aspects of the targeting, selection and screening strategies. This should be done at the earliest stage of the project as possible in order to avoid pitfalls that are common to this methodology. Details of procedures along with expectations of the *Investigator* and the TMF will be discussed during this initial consultation.
- **IVF and Embryo Cryopreservation:** Utilizing *in vitro* fertilization (IVF) of oocytes from *Investigator*-provided superovulated females (or 10 superovulated wild type females) with sperm from *Investigator*-provided males. Fertilized oocytes can be transferred to pseudopregnant recipient females or cryopreserved. Cryopreserved embryos are stored in liquid N<sub>2</sub> for up to two years free of charge. Modifications of the service such as normal mating of males to superovulated females, use of alternate mouse strains, etc., can also be accommodated by the TMF. Options are available to assess the viability of cryopreserved embryos. In some cases, additional charges may be incurred to recover mouse costs and *per diem* charges. The TMF also offers sperm cryopreservation (1-2 males per line), although a “best effort only” guarantee is in effect (i.e. sperm viability and suitability for IVF is not guaranteed). See **Section 2** for additional details.

### **CORE RESPONSIBILITIES**

- **DNA Microinjection:** The TMF will microinject a minimum of 150 fertilized C57BL/6 or B6JxSJL F2 hybrid oocytes with your DNA fragment and transfer (re-implant) them into pseudopregnant recipient females. For transgenes that are not lethal, in excess of 12 potential founder animals are expected to result per experiment, however, there is no guarantee regarding the number of mouse pups delivered. In our experience, these numbers are sufficient to generate 3-8 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 (about one week prior to weaning). **Although the TMF expects 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 TMF will inject mouse ES cells into a minimum of 40 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 40 blastocysts will be injected. This number should be sufficient to generate 4 or more 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, the TMF will schedule a second day of blastocyst microinjection at no additional charge.
- **IVF and Cryopreservation of Mouse Lines:** The TMF 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.**

- **Mouse husbandry:** All husbandry practices for TMF 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. Egg donor females and blastocyst donor females are obtained either from our internal breeding colonies or from a variety of vendors, including Taconic, Harlan and JAX. Requests for donor females to be obtained from a specific vendor will be honored when possible.

## **INVESTIGATOR RESPONSIBILITIES**

- **DNA Microinjection:** It is the responsibility of the *Investigator* to provide to the TMF an appropriately prepared DNA sample at the required concentration (please see **Section 4** for DNA preparation and concentration information) for pronuclear microinjection at the time of project submission. If necessary, exceptions to the time of submission rule are possible; however, the Coordinator 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 Coordinator 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 TMF high quality ES cells. Laboratories with experience growing ES cells should provide a clean (neither bacterial or fungal contamination, nor presence of antibacterial or antimycotic drugs) ES cell line plated at 50% confluency the day before the scheduled injection (unless other arrangements are made). ES cells generated by the TMF will be prepared for microinjection by the TMF staff. Please refer to **Section 5** 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 Coordinator is required. The *Investigator* will receive the resultant mice and their transfer recipient mothers at 2 weeks of age (1 week before weaning) for analysis of germline transmission of the genetic modification.

## **PRODUCTIVITY**

The TMF is fully operational and mice are generated using either DNA microinjection or ES cell transfer techniques. To date, the UAB TMF has generated in excess of 7500 potential genetically modified founder mice harboring transgenes or gene-targeted ES cells, and more than 100 UAB investigators have received expert assistance from, or consulted with, the TMF in their research efforts. Most injection projects are completed within 2 weeks of submission.

## **ADDING TRANSGENIC MOUSE PRODUCTION TO YOUR ANIMAL PROTOCOL**

The production of transgenic mice is covered under approved IACUC protocols to the Transgenic Mouse Facility. If the proposed transgenic animal model for which services are requested has not been previously approved under an IACUC protocol, please send an email to the IACUC ([iacuc@uab.edu](mailto:iacuc@uab.edu)) to obtain administrative approval for services to begin. This will not require full IACUC review (unless requested); however, substantial changes in total numbers of animals will need appropriate justifications.

*Example email:* I would like to modify our currently approved protocol (Animal Protocol Number: xxxxx for NIH sponsored RO1 grant entitled, xxxxxx) to include the production of transgenic mice harboring the following construct (*description*). This model will substitute for previously planned studies; therefore, we do not anticipate an increased number of total animals utilized under this protocol.

## 2. SERVICE FEES

### *Primary Services:*

Project Type	Mouse Strain	Comments	Total Fee
<b>DNA Microinjection<sup>1</sup></b>	B6xSJL F2 hybrid	150 fertilized oocytes injected	<b>\$2900</b>
	C57BL/6		<b>\$2900</b>
	Other Strains		<b>TBD<sup>3</sup></b>
<b>Gene Targeting</b>	Variety of ES cell stocks are available	No screening (DNA plates to PI)	<b>\$4500</b>
		With Southern blot screening	<b>\$6000</b>
<b>ES Cell Transfer<sup>2</sup></b>	C57BL/6 (wild type or albino) blastocysts	Inject $\geq$ 40 blastocysts / 1 clone (w/ additional clone \$300 each)	<b>\$2800</b>
	Other Strains		<b>TBD<sup>3</sup></b>

<sup>1</sup>On average, 15-20% of the mice delivered should be transgenic. For B6xSJL hybrids we anticipate approximately 25-35 mice per experiment with an average of 4-7 transgenic mice. For C57BL/6, 10-15 mice produced with 2-4 transgenic. Transgenic founder mice are not guaranteed--**this service provides a "best effort" assurance only (see Section 1)**. A \$100 non-refundable fee will be assessed if the DNA sample submitted is found to be unsuitable for microinjection. <sup>2</sup>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* will forfeit the service charge. <sup>3</sup>To be determined; additional mouse purchases and *per diem* recovery costs may be added.

### *Assisted Reproduction and Cryopreservation Services:*

<b>IVF</b>	Mice provided by PI <sup>1</sup>	Includes transfer of two-cell embryos to up to six recipient females	<b>\$1800</b>
<b>IVF/Cryopreservation</b>	Mice provided by PI <sup>1</sup>	Includes verification of pregnancy in recipient female using thawed embryos	<b>\$1800</b>
<b>Embryo Cryopreservation</b>	Mice provided by PI <sup>1</sup>	Economy (viability not tested) <sup>2</sup>	<b>\$900</b>
		Standard (test for viable pregnancy)	<b>\$1000</b>
		Premium (test pregnancy to term)	<b>\$1200</b>
<b>Transfer of cryopreserved embryos</b>		Transfer of thawed embryos to up to six recipient females	<b>\$900</b>
<b>Rederivation<sup>3</sup></b>	Mice provided by PI	Transfer of fertilized oocytes to up to six recipient females	<b>\$1000</b>
<b>Sperm cryopreservation</b>	Mice provided by PI	1-2 males per line. Motility of thawed sperm tested.	<b>\$250</b>

Prices quoted assume that female mice are provided by the *Investigator*, and there are no guarantees regarding the number of viable embryos obtained. If wild type females are used we will guarantee at least 50 two-cell embryos for transfer to recipient females or for cryopreservation, provided that sufficient fertile males are made available. A procurement charge for wild type females will be added. Cryopreservation procedures include storage for two years, after which time a fee of \$50 per year per line will apply. An additional \$200 may be paid at any time if the *Investigator* wishes to verify the viability (pregnancy to term, pups delivered to PI at two-weeks of age) of a line previously cryopreserved. <sup>3</sup>Please contact the UAB Animal Resources Program (ARP) to initiate a rederivation project.

**3. PROJECT SUBMISSION FORM - TMF – ANIMAL COMPONENT**

PI NAME: \_\_\_\_\_ DATE: \_\_\_\_\_  
DEPARTMENT: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_ EMAIL: \_\_\_\_\_  
PHONE/FAX: \_\_\_\_\_

UAB Account: \_\_\_\_\_

EXTRAMURAL SUPPORT ID/GRANT #: \_\_\_\_\_  
IACUC PROJECT APPROVAL # (APN): \_\_\_\_\_

UAB CENTER MEMBERSHIP (Check all that apply):

CCC (Comprehensive Cancer Center)       RDCC (Rheumatic Disease Core Center)  
 CNRC (Clinical Nutrition Research Center)       AMC (Arthritis and Musculoskeletal Center)  
 CMBD (Center for Metabolic Bone Disease)  
 RPKDCC (Recessive Polycystic Kidney Disease Core Center)  
OTHER: \_\_\_\_\_

In order to comply with UAB policies, the TMF requires that prior to initiating either a DNA or ES cell injection project, an investigator must demonstrate **BOTH** an updated approval by the Institutional Animal Care and Use Committee (IACUC) as well as the Institutional Biosafety Committee (IBC). For the latter, please provide the following:

***(i) please indicate the source(s) of DNA constituting the transgene to be injected (e.g. Mouse collagen promoter driving firefly luciferase reporter gene)***

***(ii) please indicate the nature of DNA sequences constituting the transgene to be injected (e.g. Human melanocortin receptor cDNA containing Arg>Ala mutation at position 300)***

***(iii) please indicate if a foreign gene will be expressed, and if so, indicate the protein that will be produced; (e.g. Human growth hormone receptor dominant negative variant)***

***(iv) please indicate the containment conditions that will be implemented as specified in the NIH Guidelines. (e.g. BL1 is appropriate for most transgenic projects, and any project needing BL2 or greater must be discussed with the Director)***

***Please note that initiation of projects is contingent upon the UAB Transgenic Mouse Facility receiving approval from:***

**Donna S. Williamson, Director IBC**  
The University of Alabama at Birmingham  
Department of Occupational Health & Safety  
933 19th St. South Suite 445 (CH19 445)  
Birmingham, AL 35294  
(205) 934-4752 or (205) 934-2487  
(205) 934-7487 fax

PROJECT NAME (12 character limit): \_\_\_\_\_

WHERE ARE ANIMALS TO BE TRANSFERRED? BUILDING/RM: \_\_\_\_\_

DNA Microinjection: Strain: \_\_\_ C57BL/6 oocytes (standard)  
\_\_\_ other (e.g. C57BL/6xSJL F2 oocytes) \_\_\_\_\_

DNA sample: estimated concentration/volume/buffer/size (kb): \_\_\_\_\_

Embryonic Stem Cell Micronjection:  
ES Cell source & Strain (e.g. KOMP gene X, C57BL/6) \_\_\_\_\_

ES Cells (mycoplasma test result, date): \_\_\_\_\_

GENOTYPING to be performed by (please check one): \_\_\_ TMF staff \_\_\_ PI staff

---

**PROJECT DESCRIPTION (include expected phenotype AND statement of potential for embryonic lethality). IMPORTANT! Your project will NOT be accepted unless this section is completed properly! (use additional pages or attachments if necessary)**

**IMPORTANT! YOU MUST INCLUDE: (1) gel photo of final aliquot for DNA microinjection, (2) restriction map specifically illustrating (including size) all elements of the transgene construct, (3) Section 6 (signed contract), and Internal Payment Form for anticipated costs. Project will NOT begin until these materials are received.**

**Box for TMF use only**

Date Received:  
Received By:  
Project Number:  
Project Name:  
Number of Cages:  
Number of Pups:  
Date of Birth:

#### **4. Guidelines for DNA Fragment Purification for Pronuclear Microinjection**

The transgene construct should be cloned in an appropriate vector (e.g., plasmid, BAC, cosmid); the DNA purified (e.g., if a plasmid, by CsCl gradient or by ion exchange column chromatography, such as Bethesda Research Laboratories NACS system, Qiagen plasmid purification kit, or other commercially available plasmid purification kits) 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 TMF for microinjection. The fragment can be concentrated using a DNA extraction protocol such as glass bead purification (e.g., GeneClean kit from [www.qbiogene.com](http://www.qbiogene.com) or Qiagen Qiaex II Gel Purification Kit is preferred), or electroelution, ion exchange chromatography, and ethanol precipitation (e.g., NACS or a Schleicher & Shuell Elutip protocols). Please exercise extreme caution during final steps of each procedure to ensure that no particulates from the final elution step (e.g. glass beads or chromatography matrix) are transferred to the aliquot that will be delivered to the TMF for microinjection. A “pure” sample, completely free of particulate matter, for pronuclear microinjection is more important than high yield.

The final transgene DNA sample should be submitted in solution in TE buffer (**10 mM Tris, 0.25 mM EDTA, pH 7.5; DNA concentration in excess of 20 ng/μl...ideally 40-100 ng/μl**) and it should be packed on ice. **IMPORTANT: The TE buffer should be sterile filtered (0.2μm) and autoclaved.** A minimum of **1 μg of purified fragment is required** for pronuclear microinjection. Specification of the sample buffer, approximate concentration, volume and size of your final DNA sample is required to appropriately dilute it for microinjection. It is strongly recommended that the investigator run out 150-200 ng of the DNA sample on an agarose gel to confirm the purity and approximate concentration before submitting to the TMF.

**Upon receiving the DNA fragment (transgene construct) TMF staff will confirm its purity and concentration using agarose gel electrophoresis. If (i) multiple bands appear, (ii) contamination is evident, or (iii) there is substantially less than 1 μg of total DNA, all remaining samples of the DNA construct shall be returned to *Investigator* with an explanation and documentation. In this case, the TMF shall not be obligated to proceed further with the experiment, and the non-refundable \$100 fee (as described in Section 2) will be forfeited.**

#### **5. Guidelines for ES Cell Preparation for Blastocyst Microinjection**

Please contact the Coordinator for scheduling of ES cell injections. The day before the scheduled injection, please provide to the Coordinator a dish of ES cells growing on feeder cells at 50% confluency. TMF staff will prepare them for microinjection the following morning. Labs with proven expertise at preparing ES cells for microinjection may be allowed to do so only with the approval of the Director or Coordinator.

**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 TMF is not obligated to proceed further and the Service Charge (described in Section 2) will be forfeited. Also, it should be understood that the quality of the ES cells provided to the TMF is the primary factor influencing production of chimeric mice. The TMF has established protocols that virtually guarantee generation of chimeric mice, *if the ES cells provided by the Investigator are satisfactory for this purpose*. The Investigator will be responsible for payment of the service charge whether or not chimeric mice are produced.**

**6. TMF TRANSGENIC MOUSE PRODUCTION SERVICE AGREEMENT**

This Agreement is made between the transgenic mouse facility (“TMF”) at the University of Alabama at Birmingham (“TMF”) and the undersigned principal investigator, an employee of TMF (the “PI”) regarding the development of transgenic and gene targeted (genetically modified) mice for the PI. This Agreement is effective as of the date of the last signature of the parties as set forth below.

**I. The Project.** TMF will (*mark one*)

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

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

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

**A.** The PI will provide TMF 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.** The PI hereby represents and warrants that (i) 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, (ii) the experiments do not require containment conditions greater than NIH RAC BL-2 standards, and (iii) to the best of the PI’s knowledge, the DNA construct(s) or cell line(s) do not infringe the intellectual property rights of any third party.

**C.** TMF shall be entitled to i) not commence its duties under Section I.A. until such time as it receives the written documentation required under Section II.A. and the initial fees or requisitions under Section III.A., and ii) terminate this Agreement immediately upon written notice to the PI, if TMF finds 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, after examination by electrophoretic gel, the PI’s DNA construct was not prepared according to the outlined guidelines or does not meet the stated requirements for microinjection. Upon such termination, the PI shall forfeit all fees paid to TMF.

**III. Fee, Payment Schedule, Terms and Conditions**

**A.** The PI shall be billed for the total estimated charges upon delivery of each DNA construct or cell line to TMF. Additional cage charges will apply (rate set forth below) if production is delayed due to the acts or omissions of the PI (e.g. incomplete paperwork).

**B.** With respect to the genetically modified mice produced pursuant to this Agreement, the PI shall (i) only use the mice for non-commercial research purposes, and (ii) not sell, lease, assign or otherwise transfer the mice or any interest therein to any third party, other than a transfer to a third party for breeding purposes only and for use solely by the PI, and to other non-profit institutions for research purposes only. The PI shall notify TMF in writing of any such transfers of the genetically modified mice.

**IV. Confidentiality**

TMF will hold in confidence the identity and nature of the PI’s projects and will limit disclosure of such matters to only TMF employees, provided however, that such confidentiality obligation does not apply to (i) information that is known to TMF on the date hereof, (ii) is or becomes public knowledge through no fault of TMF, (iii) is received by TMF in good faith from a third party lawfully in possession of such information and having no obligation to keep such information confidential, (iv) is independently developed by TMF without reference to the information, or (v) information that is required to be disclosed by applicable law or a governmental authority having jurisdiction. Upon completion or termination of TMF’s duties and obligations, TMF may retain a sample of the PI’s materials and shall, if requested by the PI, return, at the PI’s sole expense, any other remaining materials, proprietary information, cell lines or DNA constructs supplied by the PI.

**V. Miscellaneous**

**A.** TMF will provide the mice on an “AS IS” basis and the PI acknowledges that the mice are experimental in nature. TMF makes no representations or warranties of any kind, express or implied, including but not limited to warranties of merchantability, fitness for a particular purpose, successful production, or non-infringement of a third party’s intellectual property rights. The PI assumes all risks relating to the mice.

**C.** The PI agrees that any publications involving the genetically modified mice provided hereunder shall acknowledge the "TMF Transgenic Mouse Facility" as the source of the genetically modified mice. The PI shall promptly provide copies of all such publications to TMF.

**D.** This Agreement shall be construed in accordance with the laws of (i) the State of Alabama and (ii) applicable policies of TMF and UAB.

IN WITNESS WHEREOF, the duly authorized representatives of the parties have executed this Agreement.

<b>TMF</b>	<b>The PI:</b>
By:	By:
Name:	Name:
Title:	Title:
Additional rate per cage per day: \$	