

UNIVERSITY OF ROCHESTER TRANSGENIC FACILITY INSTRUCTIONS TO USERS

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Please note: Sections 3, 4 and 8 along with an internal requisition and accompanying documentation are all required at the time of each project submission. Many of the services that are offered by our Facility are performed for research purposes only – due to a number of patent-related restrictions (all of the processes used for creating genetically-engineered mice are patented by other entities). The models and biological materials (e.g., cells, nucleic acids, antibodies, etc.) derived from them may not be distributed to third parties or used for commercial purposes without proper consent. Should you have any concerns or questions about the intellectual property rights in relation to your research, please contact the Medical Center Office of Technology Transfer at 784-8850 (for Medical Center-based projects), or the University of Rochester Office of Technology Transfer at 275-3998 (for River Campus-based projects).

Last Revised: June 1, 2005
Dr. C.A. Pinkert, Director

1. PRIORITIES, SERVICES AND PRODUCTIVITY

PRIORITIES

Initially, the UR Transgenic Facility (URTF) services have included the production of mouse models using DNA and embryonic stem cell injection methodologies. The services of the URTF are available to UR investigators on a first come basis. Scheduling of work is determined by the Director. If assignment of priorities by which investigators will be served becomes necessary, the Director will be assisted by the URTF Advisory Committee: Drs. M.B. Taubman (Chair, Cardiology, CCVR), A. Bottaro (Medicine), J.G. Frelinger (Micro. and Immunol.), L. Gan (Ophthalmology, CADB), R. Jiang (Biomed. Genetics, COB), M.K. O'Banion (NBA), R.H. Pierce (Pathology), C.A. Thornton (Neurology), D.J. Topham (Micro., CVBI), and *ex officio* members: D. Moorman-White (DLAM), B.C. Vanwuyckhuysse (SMD), and C.A. Pinkert (URTF).

An investigator may submit any number of constructs or cell lines annually. At this time, priority among applicants for services shall be established according to the following criteria: date of application and reagents (including receipt of DNA construct, targeting vector or ES cells); status as part of a funded research project; number of transgenic animals previously created by the URTF for the investigator; relevance of the transgenic model to funded research aims at UR, and other criteria as shall be identified and set by the URTF Advisory Committee.

SERVICES

Introduction

There will be six primary services offered to UR Investigators (see page 4 for pricing).

- a) **DNA Microinjection:** 150 ova are injected per "project". Comprehensive service including genotyping and a 3 transgenic mouse guarantee (with putatively non-lethal transgenes) is provided. At this time, B6SJLF2, ICR, C57BL/6 or FVB genetic backgrounds can be accommodated and 12 or more offspring will be generated for analysis by the User, or a 2nd round of injection will be performed. Other strains can be accommodated as noted on page 4.
- b) **ES Cell Transfer:** 20-40 blastocysts are injected with a User's ES cell clone per "project"; 12 or more offspring or 3 (>50%) chimeras will either be delivered or the cell line will be reinjected.
- c) **Gene Targeting:** A comprehensive gene-targeting service includes electroporation of a User's vector, clone selection/propagation/analysis (Southern or PCR), and chimera production.
- d) **Line Rederivation** (using donor males in IVF). Costs are based on superovulating 12 females*, with fertilized ova transferred to a maximum of 3 synchronized recipients. Health surveillance data will be obtained prior to delivery.
- e) **Embryo Cryopreservation** (using donor males in IVF). Fifteen females* are superovulated, with resultant ova that are fertilized stored for up to one year. Embryo recovery, culture, and transfers within one year to a maximum of 3 recipients are included in this service.
- f) **Sperm Cryopreservation** (using 2-4 donor males). Mouse spermatozoa will be harvested and cryopreserved. Chargeback for any test IVF protocols is not included.

*Procedures are established using the most cost-effective strains to maximize overall efficiencies. Individual inbred and "non-standard" strain requirements may result in higher expenses (see page 4).

Investigator Responsibilities

In order to provide transgenic mice to the investigator, either a linearized DNA fragment (for microinjection) or transfected cell line (for ES cell work) is required. Projects requiring a containment level greater than NIH RAC BSL-2 cannot be accommodated. For projects requiring BSL-2 containment, Institutional IBC approval is required. Detailed information related to DNA Fragment and ES Cell Preparation Guidelines can be found in Sections 5 and 6, respectively.

URTF Responsibilities

DNA Microinjection:

The URTF will microinject fertilized mouse eggs with an Investigator's DNA fragment and reimplant surviving eggs into pseudopregnant recipient females. On average (and strain dependent), these numbers should be sufficient to generate 3-5 founder transgenic mice. Founder transgenic mice (and littermates if desired) will be shipped to Investigators, following weaning and genotyping.

ES Cell Injection:

The URTF will inject your stem cells into 2½ to 3½ day mouse morulae or blastocysts or perform co-culture procedures with 2½ day morulae and reimplant eggs into pseudopregnant recipient females. These procedures will be performed on a "per day" basis with the expectation that a minimum of 20-40 blastocysts will be injected, or 50 morulae co-cultured or injected per day. This should be sufficient to generate founder chimeric mice. All new ES cell lines will have mycoplasma testing data provided at submission. Cell clones will be karyotyped (chromosome counting only) as part of our standard service.

In addition to primary services, as noted on page 4, expanded embryonic stem cell and gene knock-out mouse model assistance is also available. ES cell gene targeting services include: **1)** Transfection of PC1 (129S6/SvEvTac, generated in the URTF), AB1 (129S6/SvEvTac, from Dr. A. Bradley), or E14.1 (129P2/Ola, from Drs. R. Kuhn and K. Rajewsky) ES cells with investigator-supplied targeting vectors. **2)** Positive selection performed under G418 (neomycin) or hygromycin B pressure performed and if desired, negative selection (e.g., using gancyclovir), followed by **3)** Propagation of clones in duplicate for further analysis. **4)** We will analyze clones by Southern blot (or PCR-based screening) for identification of targeted ES cell clones with Investigator supplied probe (or primers). **5)** Targeted clones will be expanded, reconfirmed, and injection of 1 or 2 separate clones is then performed to produce founder chimeric mice (additional rounds of injection will be subject to fees outlined for primary services on page 4). Reagents currently available for ES cell culture include: ES cell-qualified fetal bovine serum or synthetic sera, homozygous neomycin-resistant mouse embryonic feeder cells (MEF cells, passage 2 or 3), MEF cells ± pretreatment with mitomycin-C, a 129P2/Ola library, and E14.1 (129P2/Ola) ES cells. In addition, 129S6/SvEvTac ES cells and an isogenic CHORI RPCI-22, 129S6 mouse BAC library (high density filters – available on request from the URTF) are provided on a cost-recovery basis.

PRODUCTIVITY

The URTF is fully operational and mice (on various genetic backgrounds) are generated using either DNA microinjection or ES cell transfer techniques. Additional services have included: cryopreservation of embryos, rederivation of pathogen-free mice, and assisted reproduction techniques (e.g., *in vitro* fertilization [IVF], superovulation, and embryo transfer).

Only animals from approved sources, known to be free of pathogens based on health surveillance test results, are allowed entry into the URTF barrier colony. All husbandry practices for URTF animals involve those of strict barrier maintenance. Ventilated bioexclusion caging is used. Cages are changed only in laminar flow change stations by trained personnel wearing protective garb. Cages, food, bedding, water bottles, etc., are autoclaved into the URTF barrier. Personnel working in the barrier are not allowed contact with other lab animals or access to other animal areas.

2. COSTS (effective: July 1, 2005)

Primary Services	Minimum Guarantee	Mouse Strain*	Cost
DNA microinjection – comprehensive	150 eggs injected; PCR genotyping; 3 transgenic mice delivered ^{a, b}	B6SJL hybrid	\$2500
		C57BL/6 or ICR	\$3500
		FVB inbred	\$4000
ES cell transfer	inject 20-40 blastocysts; 12 pups or 3 (>50%) chimeras delivered ^c	C57BL/6 or ICR	\$2500
<i>Gene targeting</i>	see below ^d		\$7500
Line rederivation/IVF	None	B6SJL hybrid	\$2000
<i>Embryo cryopreservation/IVF</i>	None	B6SJL hybrid	\$2000
<i>Sperm cryopreservation</i>	None ^e		\$ 750

^a For a 3 transgenic mouse guarantee, putatively non-lethal transgenes are included here. If two transgenic mice are not identified after the 2nd round of injection, as noted above, only \$750 will be due.

^b A \$150 non-refundable fee will be assessed if a DNA sample is found to be unsuitable for microinjection.

^c If 12 live mice or 3 chimeras are not produced after initial injections, the cell line will be reinjected. The project is considered completed in full at that time.

^d For ES cell services – beginning with the Transgenic Facility’s gene targeting efforts using a PI’s vector, through to production of founder mice (one round of injection), the cost will be \$7500 (reflecting a cost savings over reduced individual component costs). Charges accruing through individual production steps: 1) \$2500 – electroporation and clone selection, 2) \$2500 – Southern blotting and clone propagation, and 3) \$2500 – injection of one or two ES cell clones.

^e Excluding any test IVF procedures and/or transfers; cryostorage costs accommodated through 1 year.

* Work with other strains will require additional mouse charges and per diem recovery. For DNA microinjection using strains other than B6SJL hybrid, C57BL/6, FVB and ICR, **OR** for ES cell transfer into strains other than C57BL/6 or ICR, the FVB inbred charges **plus the purchase cost of mice** will apply with no guarantees (i.e., setup for one day only, if egg yield less than 150 eggs for DNA microinjection or less than 20 eggs for ES cell transfer, costs will be incurred as outlined and the particular “project” will be considered completed).

Other services/charges:

Item	Cost	Minimum Charge
Blood sampling/other biopsies	\$10/sample (or per tissue)	\$50
Tail sampling: 3-4 weeks of age	\$5/sample	\$50
Tail sampling: over 4 wks of age (analgesia/anesthesia required)	\$10/sample	\$50
PCR analyses	\$25/sample (incl. biopsy)	\$300
Dot/slot blot DNA analysis	\$50/sample (incl. biopsy)	\$300
Southern blot analysis	\$75/sample (incl. biopsy)	\$500
Mycoplasma testing	\$125/cell line	\$125
Mice: Sentinels – outbred Swiss/ICR	\$ 5.00 per mouse*	\$25 per order
Males, or hybrid/inbred strains	\$15-\$20 per mouse*	\$25 per order
Newborn pups	\$50 per litter minimum*	\$50
Time-mated pregnant C57BL/6 inbred females (time-mated females >E13 with pregnancy confirmation, \$80)	\$50-80 each*	\$50
Lactating females (± pups) and time-mated ICR females (time-mated females >E13 with pregnancy confirmation, \$60)	\$35-60 each*	\$35
*Note: If necessary, mice may be shipped in sterile, filtered, and disposable Transit Boxes at a cost of \$10–\$25/box.		
Per Diem (full cost-recovery – including Vivarium recharge):	\$.30/mouse/day	\$25/week

3. PROJECT SUBMISSION FORM*

UR Transgenic Facility

DATE: _____

NAME: _____

DEPARTMENT: _____

ADDRESS: _____

PHONE/FAX: _____

E-MAIL: _____

UR ACCOUNT: _____

EXTRAMURAL SUPPORT ID/GRANT #: _____

UR CENTER MEMBERSHIPS: _____

UCAR PROJECT APPROVAL #: _____

WHERE ARE ANIMALS TO BE HOUSED?: _____

PROJECT NAME (12 character limit): _____

STRAIN/SPECIALITY REQUIREMENTS: _____

DNA Microinjection: _____

(DNA fragment length/concentration/volume/buffer)

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

PROJECT DESCRIPTION: _____

***PLEASE ATTACH: 1.) gel photo of final aliquot for DNA microinjection projects or Southern confirmation for gene targeting projects, 2.) restriction map (including fragment length and 3' & 5' cutters), 3.) Sections 4 and 8, and other requested materials as noted, and 4.) a requisition for anticipated costs.**

UR TRANGENIC FACILITY USE ONLY BELOW THIS BOX

Date Received: _____ By: _____ Project # _____

revised: 30 November 2004

4. UR Mouse Transfer Information

[on receipt this page will be forwarded to the UR Vivarium by the UR Transgenic Facility]

REQUEST FOR ANIMAL PROCUREMENT

University of Rochester Vivarium
 School of Medicine and Dentistry, Box 674
 voice 5-2651 • fax 3-1085/3-2490

DATE OF REQUEST: _____

UR ACCOUNT NUMBER: _____

UCAR APPROVAL NUMBER: _____

HOUSING ASSIGNMENT: _____

INVESTIGATOR: _____

DEPARTMENT: _____ EXT. _____

LAB CONTACT: _____ EXT. _____

VENDOR: UR TRANSGENIC FACILITY

DATE NEEDED: WHEN AVAILABLE

QUANTITY	SPECIES/DESCRIPTION	WEIGHT	SEX	AGE
	ICR embryo transfer recipients with 2 week old litters		♀	10-16 wks

SPECIAL REQUIREMENTS:

URTF/UR VIVARIUM BUSINESS OFFICE USE ONLY BELOW THIS BOX

URTF Project #: _____ Vivarium Animal Ordering Req. #: _____

Date form 1st received in Vivarium: _____ By: _____

Vivarium approval (circle) **yes no** Date: _____ By: _____

Comments: _____

Shipping date intermediate: _____ (identified by Friday of prior week). Verified: _____

Shipping date intermediate: _____ (identified by Friday of prior week). Verified: _____

Shipping date final: _____ (identified by Friday of prior week). Verified: _____

Project Completed: _____

5a. DNA MICROINJECTION: REQUIRED GUIDELINES FOR DNA FRAGMENT PREPARATION AND ANALYSES

DNA Fragment

Initially, we recommend that you perform appropriate in vitro expression assays prior to submission of each project. The gene or genetic construction of interest should be cloned into an appropriate vector (e.g., plasmid or cosmid); the vector purified (e.g., if a plasmid, by CsCl gradient or by ion exchange column chromatography [e.g., a Bethesda Research Laboratories NACS system]); the vector digested with appropriate restriction enzyme(s) to remove all or most extraneous (e.g., prokaryotic) sequences; and, the linear fragment to be microinjected isolated by gel electrophoresis and concentration/purification.

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 absolutely free of particulate matter.

The fragment may be submitted **after ethanol precipitation, in solution – in microinjection-specific TE buffer (10 mM Tris, 0.25 mM EDTA, pH 7.5 – the DNA concentration should be approximately 100 ng/μl; minimally it must be submitted in excess of 25 ng/μl).**

Approximately 1 - 3 μg of isolated fragment is required. Specification of the exact concentration and molecular weight of your DNA is requested to enable appropriate dilution for injection.

Upon arrival of the DNA construct (DNA fragment), UR Transgenic Facility staff will take an aliquot of the DNA construct and, using gel electrophoresis, confirm purity and concentration of the DNA construct. If (a) multiple bands appear, (b) contamination is evident, or (c) if there is less than 1 μg of total fragment DNA, all remaining samples of the DNA construct shall be returned to Investigator with explanation and documentation, and the UR Transgenic Facility shall not be obligated to proceed further (and the non-refundable fee as described on page 4 will then be forfeited).

PCR

For identification of founder transgenic mice, either PCR or DNA hybridization (e.g., dot blot) analyses can be performed. If PCR analysis is to be performed, it is suggested that the Investigator obtain appropriate oligonucleotides and specifications of proven conditions for the reactions.

Hybridization Probe

The Facility recommends that the investigator prepare 5 to 100 μg of a plasmid containing the gene or other appropriate sequences to serve as a hybridization probe for identification of founder transgenic mice. The vector/probe should be known not to hybridize to mouse DNA under conditions of reasonable stringency, and probes should be labeled in a sufficient timeframe relative to the experiment.

5b. DNA MICROINJECTION: REQUIRED GUIDELINES FOR PCR ANALYSES

If PCR analysis is to be used to identify transgenic mice, we recommend appropriate oligonucleotide primers and specification of proven conditions for the reaction. If needed, we will provide mouse DNA (B6SJL F1 hybrid, C57BL/6, or FVB as appropriate) for evaluating PCR specificity. You should complete the information below and if desired, include photographs (originals or discernable copies) of the control reactions with your application and your protocol/results will be reviewed upon submission.

5' primer name (≤ 12 characters) _____

5' primer length (bp), molar conc., total conc. _____, _____, _____

3' primer name (≤ 12 characters) _____

3' primer length (bp), molar conc., total conc. _____, _____, _____

Length of PCR product (bp): _____

Amount of each primer per reaction (μl): 5' _____ 3' _____

Denaturing Temp. ($^{\circ}\text{C}$): _____ Denaturing Time: _____

Annealing Temp. ($^{\circ}\text{C}$): _____ Annealing Time: _____

Extension Temp. ($^{\circ}\text{C}$): _____ Extension Time: _____

Cycles: _____

Any additional pertinent information regarding reaction conditions: pH _____, MgCl_2 conc. _____, Taq conc. _____, dNTPs _____, KCl conc. _____, other _____

Reaction conditions - all primers must be tested before submission (with controls) as follow:

1. Normal mouse DNA (designated NM; use the specific mouse strain appropriate to your project)
2. NM + 0.1 (or 0.5) gene copy/cell equivalent of the DNA construct
3. NM + 1 gene copy/cell equivalent of the DNA construct
4. NM + 5 (or 10) gene copies/cell equivalent of the DNA construct
5. 1 gene copy/cell equivalent of the DNA construct (no NM DNA)
6. 5 (or 10) gene copies/cell equivalent of the DNA construct (no NM DNA)
7. Appropriate marker DNA

[Genomic Conversion for DNA copies per cell - 6×10^9 base pairs per diploid genome.]

Marker DNA (lane 7 above): Type: _____ Concentration: _____ Volume (μl): _____

Reaction Volume (μl): _____

Amount of mouse DNA per NM reaction (ng): _____

Amount of DNA used in 1 copy control (pg): _____

[ATTACH A GEL PHOTO OF YOUR PCR REACTION.]

5c. DNA MICROINJECTION: RECOMMENDED GUIDELINES FOR DNA HYBRIDIZATION ANALYSES

If nucleic acid hybridization is to be used to identify transgenic mice, generally 1 µg of purified linear probe fragment (the “probe”) containing the gene or other appropriate sequences to serve as a hybridization probe is required. The probe should be isolated in the same manner as the DNA construct used for microinjection, with the exception that following gel elution, column purification is not required. The probe must be sufficiently pure to be labeled by standard techniques.

The probe should not hybridize to normal mouse DNA under conditions of reasonable stringency. If needed, we will provide mouse DNA (C57BL/6 x SJL F1 hybrid, C57BL/6 or FVB as needed) to evaluate putative probes. Our method of analysis does not separate endogenous hybridization signals from transgene signals. If Southern blot is required to distinguish endogenous genes (normal C57BL/6, SJL, or FVB mouse DNA) from transgenes, be aware that sensitivity may not be sufficient to identify mosaic founders. One should complete the information below prior to submitting a project, if these hybridization analyses are preferred. If you include photographs of the probe fragment and autoradiographs/images of the control information (originals or discernable copies), your protocol/results will be reviewed upon submission.

Probe name (≤ 12 characters): _____

Probe length (bp): _____

Probe stock concentration (ng/µl): _____

Method used to purify probe: _____

Hybridization buffer (and temperature): _____

Wash protocol: _____

The probe should be dissolved in TE buffer (pH 7.5). Please include a photograph of the probe fragment on an agarose gel with the appropriate markers (length/quantitative markers).

Samples to run per well/lane as follow:

1. Normal mouse DNA (designated NM; use the specific mouse strain appropriate to your project)
2. NM + 0.5 gene copy/cell equivalent of the DNA construct
3. NM + 1 gene copy/cell equivalent of the DNA construct
4. NM + 5 (or 10) gene copies/cell equivalent of the DNA construct
5. 1 gene copy/cell equivalent of the DNA construct (no NM DNA)
6. 5 (or 10) gene copies/cell equivalent of the DNA construct (no NM DNA)

[Genomic Conversion for DNA copies per cell - 6×10^9 base pairs per diploid genome.]

[IF DESIRED, ATTACH A GEL PHOTO OF YOUR HYBRIDIZATION RESULTS.]

5d. DNA MICROINJECTION: CALCULATING SINGLE GENE COPY/GENOME EQUIVALENT

For analyses in Sections 5b. and 5c., calculation of copy number controls would be needed. A simplified calculation to assist you in identifying the amount of construct-specific DNA for your controls follows. Keep in mind that if whole vector (e.g., plasmid) is used to spike normal mouse DNA (NMDNA), that one should use a ratio of construct to vector in the final calculation.

The mouse diploid genome has a mass of 6.42×10^{-12} g

The amount of NMDNA used in the assay (e.g., PCR or Southern) divided by this number gives the equivalent number of mouse diploid genomes. If, for example, a PCR assay was set up with 100ng mouse DNA per sample,

$$100\text{ng} = 1 \times 10^{-7}\text{g}$$

$$\frac{1 \times 10^{-7}\text{g}}{6.42 \times 10^{-12}\text{g/diploid genome}} = 15,576 \text{ diploid genomes}$$

The size of the construct in bp multiplied by 1.07×10^{-21} g/bp = the mass of the construct in grams.

So, the mass of the construct multiplied by the number of diploid genome equivalents = the single gene copy equivalent. For example, if the construct is 5,000 or 10,000 bp and we are using 100ng of DNA in our PCR, then the mass of the construct is:

$$5,000 \text{ bp} \times (1.07 \times 10^{-21} \text{ g/bp}) = 5.35 \times 10^{-18} \text{ g}$$

$$10,000\text{bp} \times (1.07 \times 10^{-21} \text{ g/bp}) = 10.70 \times 10^{-18} \text{ g}$$

Therefore for 100ng (or 1.5576×10^4 genome equivalents), the single gene copy/cell equivalent is:

$$\text{For } 5,000 \text{ bp: } (5.35 \times 10^{-18} \text{ g}) \times (1.5576 \times 10^4) = 8.33 \times 10^{-14} \text{ g or } 0.0833 \text{ pg}$$

$$\text{For } 10,000\text{bp: } (10.7 \times 10^{-18} \text{ g}) \times (1.5576 \times 10^4) = 16.67 \times 10^{-14} \text{ g or } 0.1667 \text{ pg}$$

6. REQUIRED GUIDELINES FOR ES CELL PREPARATION

[for each day of injection – if cells prepared by the PI's laboratory (and not by the Facility)]

We require mycoplasma testing of all cell lines (e.g., Molecular Probes M-7006 mycoplasma test kit), and results of analyses must accompany all submissions. Additionally, aneuploidy/euploidy analysis of cells lines is recommended prior to submission. For information regarding counting of ES cell chromosomes, or should other questions regarding methodology arise, please contact the Facility. If new/novel cell lines are to be used, we also recommend testing such wild-type cells by embryo injection before proceeding to transfections.

The ES Freezing medium contains ESC Medium (DMEM with high glucose [4.5%]; JRH Biosciences, Lenexa, KS with FBS) and DMSO.

- ESC Medium (DMEM containing 4.5% glucose)
- 15% FCS or FBS (heat inactivated, final concentration is 15%)
- (Also, pen-strep, 200mM L-glutamine, 2-mercaptoethanol and LIF)
- 10% DMSO

Keep Freezing medium cold (on ice).

Freeze $\sim 5 \times 10^6$ cells/1 ml ampule or 2 ml cryovial.

Seed: 6×10^6 ES cells/P100 for 2 day culture

4×10^6 ES cells/P100 for 3 day culture

8 - 12×10^6 for 1 day culture.

Refeed plates daily.

[note: These cells are grown on primary feeders.]

As a general rule, thaw cells at least 3 days before injection experiment is planned.

We normally use 1 - 2 ml 0.05% trypsin to detach cells before injection.

On the day of injection, bring 1 ml containing 1×10^6 ES cells in injection medium [ESC Medium, 15%FCS + 20 mM HEPES]. Also, please bring 25 ml of injection medium in a separate tube(s) if one or more days of injection are planned in a given week.

Keep cells on ice and have ready at 9:00 a.m.

Alternatively, vials of frozen ES cell stocks can be provided to the URTF at the time of project submission.

7. REQUIRED GUIDELINES FOR GENE TARGETING EXPERIMENTS

Investigator Checklist (include a copy of this checklist with vector submission).

- 1) Provide 50 – 60 μg DNA in 0.1X TE (1 mM Tris, 0.1 mM EDTA, pH7.5) at a conc. of 1 $\mu\text{g}/\mu\text{l}$ (note: linearized DNA preferred).
- 2) Method used to purify DNA (CsCl purification recommended): _____.
- 3) Length of targeting vector homology: short arm, _____ kb; long arm, _____ kb.
(note: minimum recommended total homology, 1 kb for short arm, 3 kb for long arm).
- 4) Mouse strain used to develop targeting vector (please be specific): _____.
- 5) Amount of DNA deleted as a result of recombination: _____ kb
- 6) Selection information:
 - Neomycin resistance: Yes / No
 - Hygromycin resistance: Yes / No
 - Thymidine kinase: Yes / No
 - Diphtheria toxin: Yes / No
 - Other: Yes / No (if Yes, please describe: _____)
- 7) Screening information:
 - PCR Yes / No (if Yes, provide information outlined in Section 5b, including primers, PCR conditions, and approximate expected product size)
 - Southern blot Yes / No (if Yes, provide information outlined in Section 5c, including probe, approximate expected band size, and restriction enzymes required for digests)
- 8) **Provide a photo of assay confirmation** (i.e., PCR and/or Southern data demonstrating identification of amplicon or wildtype band).

8. UR TRANSGENIC FACILITY TRANSGENIC MOUSE PRODUCTION SERVICE AGREEMENT

This Agreement is made between the UR Transgenic Facility (herein, "Facility") at the University of Rochester (UR) and _____ (herein, "PI") for the purposes of the development of transgenic mouse models for the PI.

I. The Project The Facility will (circle a, b or c): (a) microinject DNA fragments (construct/s) into _____ (strain) mouse eggs to create transgenic mice, (b) transfer ES cells into C57BL/6 embryos to create chimeric mice using the PI's ES cell clones, or (c) Other - _____.

A. Facility responsibilities: We will use reasonable efforts to create founder transgenic mice through (a) microinjection of DNA into mouse embryos (zygotes) using the DNA construct supplied by PI, (b) transfer of cell lines supplied by PI using mouse embryos, or (c) as defined above.

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

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

A. PI will provide the Facility 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 experimentation does not require containment conditions greater than those required under NIH RAC Biosafety Level 2 standards. For BL-2/BSL-2 projects, Institutional approval must be obtained by the PI prior to submission. All projects involving the *de novo* generation of transgenic animals defined as any modification of the animal's genome using recombinant DNA technology must be registered with the Institutional Biosafety Committee <http://www.safety.rochester.edu/ibc/ibcproc.htm>. All other proposals for transgenic rodent generation will be considered by the IBC as Risk Group 1, and IBC registration can occur simultaneously with experiment initiation. Projects requiring a containment level greater than BL-2 as defined by the NIH Guidelines for Recombinant DNA cannot be accommodated.

C. Institutional Animal Care and Use Committee (UCAR) approval is required before project initiation involving any animals.

D. The Facility shall be entitled to (i) not commence its duties under Section I.A until such time as it receives the written documentation required under Sections II.A., II.B. and II.C. and initial fees or requisitions under Section III.A. and (ii) 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 of an electrophoretic gel, that PI's DNA construct was not prepared according to the outlined guidelines or does not meet the stated requirements for microinjection (a specified fee will be forfeited as identified on Page 4 of this document).

III. Fee, Payment Schedule, Terms and Conditions

A. PI shall provide a valid 312 requisition for total estimated charges upon project initiation and DNA construct or cell line delivery.

B. PI shall use the transgenic mice provided under this contract solely for purposes of non-commercial research. PI shall not use the transgenic mice other than as provided herein and shall not sell, lease, rent, barter away or otherwise transfer the transgenic mice or any interest therein, except that transgenic mice may be transferred to a third party for purposes of having the third party breed the transgenic mice solely for use by PI and, except that transgenic mice may be provided to other institutions for research purposes. The Facility will be notified by PI of any such transfers of animals.

IV. Confidentiality

A. The Facility will hold in confidence the identity and nature of PI's projects and will limit disclosure of such matters, provided however, that such confidentiality obligation does not apply to (i) information that is known to the Facility on the date hereof or becomes known to the Facility 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 Facility's duties and obligations, the Facility 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 any mice from the Facility, 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 and to defend and indemnify the Facility against 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 Facility's use of said materials as contemplated herein or the use of any transgenic mice by PI or by any third party who obtains such transgenic mice from PI or (iii) the use, storage, handling, distribution, or disposal of any transgenic mice by PI or by any third party who obtains such transgenic mice from PI, to the extent provided under law.

C. PI hereby agrees that any scientific publications involving transgenic mice provided hereunder shall acknowledge the "UR Transgenic Facility" as the source of the transgenic mice. PI shall promptly provide copies of all such publications to the Facility.

D. PI hereby acknowledges that although the Facility will use all reasonable efforts to produce transgenic mice, it is not possible for the Facility to guarantee the successful production of transgenic mice hereunder and that the Facility 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 (i) the State of New York and (ii) UR regulations.

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

THE FACILITY

THE PI

By (signature): _____

By: _____

Name: _____

Name: _____

Date: _____

Date: _____