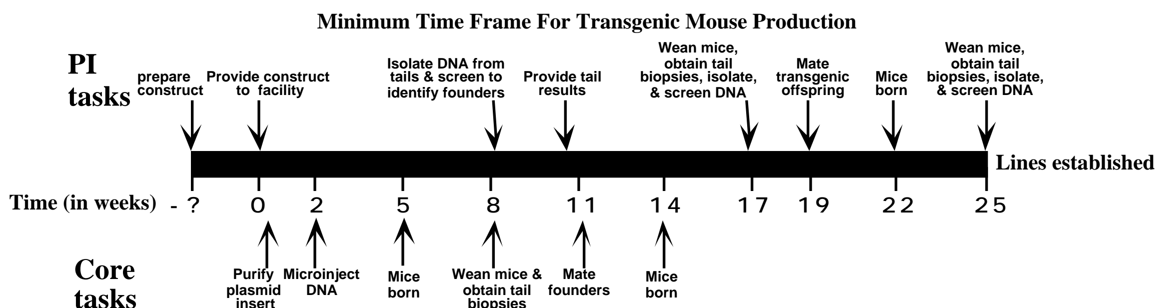
Steps required for generating transgenic mice

1. Time course. Several steps are required to generate transgenic mice. The investigator must first design a transgenic construct with a promoter and a gene (for example a reporter gene such as lacZ or a cDNA expressing an oncogene). Following preparation of plasmid DNA by the investigator, the core facility will purify the plasmid insert and microinject this DNA into fertilized mouse eggs. Potentially transgenic mice are born. Tail biopsies are given to the investigator so that they can isolate DNA for analyses by Southern blot or PCR, resulting in the identification of transgenic founders. Transgenic founders are bred to produce transgenic offspring. Core personnel are available for consultation at all stages in the development of transgenic mouse models. The minimum time frame for the generation of transgenic mouse lines is shown below. Contact Jeffrey Ceci (Ph. 409-772-2811, Email: jceci@utmb.edu) with any questions.



2. Experimental design. What is the purpose of your experiment? Do you want to try to identify tissue specific regulatory sequences? Do you want to overexpress a protein in a specific cell lineage? Do you want to be able to induce the expression of a particular protein? You will need to obtain a clone of the appropriate promoter and the gene. Expression of some transgenes may cause embryonic lethality. If you anticipate that transgene expression may be embryonic lethal, please inform us on the service request form. Appropriate expression of a transgene requires that the appropriate regulatory element(s) be included in the DNA construct. We recommend using well-characterized promoters that have previously been used to generate transgenic mice. In cases where well-characterized promoters are not available, we recommend preliminary studies in cell culture to test that the construct can be expressed in the appropriate cell type(s). Even when such studies have been conducted, it is not always possible to predict in advance whether the transgene will be expressed appropriately *in vivo*. Commercially available vectors that may be useful in transgenic research include: 1) the CMV-IE promoter for widespread gene expression, 2) tetracycline, RU-486, or ecdysone regulated gene expression systems for inducible gene expression, 3) ß-galactosidase, 4) luciferase, and 5) green fluorescent protein reporter genes. My laboratory has expression vectors with the

following promoters: Lck (thymocyte specific expression), immunoglobin promoter/enhancer (lymphoid specific expression), and ß-actin (ubiquitous expression).

3. Cloning and verifying the transgene. When designing the construct, the investigator must be aware of several potential pitfalls. For example, prokaryotic vector sequences can interfere with the expression of some transgenes; thus, unique restriction sites at the 5' and 3' ends of the construct should be available for vector removal. The transgenic construct should contain unique sequences so that its presence can be easily distinguished from the endogenous gene and so that transgene expression can be distinguished from endogenous expression. Junction fragments should be sequenced to confirm that the construct has a functional promoter, initiation codon, and polyadenylation signal. Ideally, the transgenic construct is tested for expression *in vitro* (i.e. a tissue culture system) before transgenic mice are made. If your laboratory does not wish to perform the construction of the transgene, the Recombinant DNA Core Laboratory (directed by Dr. Tom Wood, Ph. 70387) is able to perform this service.

Additional considerations when designing a construct:
1. Inclusion of a strong polyadenylation site (such as the SV40 polyadenylation sequence)
2. Make sure that you have a method (i.e., appropriate restriction endonuclease sites) of cutting out the insert of the construct, without disrupting the insert.
3. Use a well-characterized promoter, one that should be expressed in the appropriate tissues and at the correct time in development. Promoters that have not been well-characterized may result in unexpected surprises!
4. Include an intron in the construct so that the transcript will be spliced. Splicing is thought to be important for getting sufficient levels of transgene expression.

My laboratory has a plasmid whose insert contains a SV40 polyadenylation signal and an intron.

4. Establishing a screening method. You will need to establish a Southern blot or PCR assay to rapidly identify transgenic mice. The ideal probe for Southern analyses is one that is not normally present in the mouse genome (such as the SV40 polyadenylation sequence). Before you submit your DNA for microinjection into fertilized mouse eggs, the Core requires that you have a PCR or Southern blot assay that detects your transgene. We also strongly recommend a Southern blot assay so that you can determine the copy number and transgene integrity in the transgenic founder mice prior to breeding.

5. Establishing an expression assay. This may involve establishing an antibody assay to an overexpressed protein (Western blot, radioimmunoassay, immunohistochemistry, etc.), a RT-PCR assay to detect transgene transcripts, a Northern blot, or a RNase protection assay.

6. DNA purification and microinjection. We will isolate the DNA insert from the plasmid construct. If the investigator wishes to perform this step, we will provide an approved protocol for the purification of the insert of the DNA construct. You are required to verify that you have used our approved protocol. If we are not able to microinject your DNA (due to injection needles clogging), we will ask you to repurify your construct.

7. Screening potential founders. Six weeks (3 weeks gestation time and 3 weeks of post-natal growth) after microinjection, we will obtain tail biopsies from potentially transgenic mice. The investigator will extract DNAs from the tail biopsies (see protocol for "DNA from mouse tails" on our web page) and test the DNAs for the presence of the transgene by Southern blot analyses (see protocol for "Southern blots" on our web page) or PCR.

8. Southern analyses. Prior to breeding founders, we strongly recommend Southern blot analyses to verify transgenic status, to determine the approximate transgene copy number, and to determine if the transgene is intact. Occasionally transgenes may integrate in multiple chromosomal sites. Southern analyses from offspring of founders may reveal if this occurs (In such cases, you may see a difference in copy number or a different banding pattern in different offspring obtained from breeding the founder).

9. Breeding and analysis of transgenic mice. The final step in the process is to establish the transgenic line by breeding the founder. Typically, the transgenic founder animals are bred to mice of a defined genetic background such as C57BL/6. Once the offspring have reached 20 days of age, maintenance of the mice will become the responsibility of the investigator. At the time of weaning, my technicians will conduct an orientation to the investigator's staff (if needed) about how to collect tail biopsies, and how to maintain transgenic lines. If the investigator wishes, the facility will maintain mouse stocks for $26.25/hr[1]. This service will be available as long as the facility is not overwhelmed with maintaining too many transgenic stocks. In this case, priority will be given to the newest transgenic stocks and to collaborative projects. For recommendations how to maintain lines, see "Maintenance of transgenic/knockout stocks" in the protocols section of our web page.

10. Analyzing expression. Offspring of founders should be analyzed to determine whether they express the transgene. This analysis should be conducted as early as possible to minimize cage costs. Expression of the transgene is usually dependent upon the site of integration of the transgene. There is not generally any correlation between transgene copy number and expression of the transgene. Lines that do not express the transgene should be eliminated. There is not really anything that can be done to control which lines express the transgene and which line do not express the transgene. Generally, 2 lines that express the transgene should be maintained for future experiments. Of course, more could be maintained, but this would entail greater maintenance costs.

COMMONLY ASKED QUESTIONS

What services are offered by the Transgenic Core?
(1) Microinjection of DNA into the pronucleus of fertilized eggs to generate transgenic mice.
(2) Isolation of embryonic stem (ES) cell lines that have targeted mutations (knockout cell lines) by electroporation of DNA into ES cells.
(3) Microinjection of ES cells into mouse blastocysts to generate knockout mice.
(4) Rederivation of specific pathogen free mice from pathogen infected mice.
(5) Cryopreservation of mouse embryos and mouse sperm for investigators who wish to stop breeding a line or to preserve a mouse line from accidental loss.
(6) Regeneration of a mouse line from cryopreserved embryos or sperm.
(7) Special services: Isolation of DNAs from tail biopsies of founders, screening for transgenic founders by Southern hybridization, maintenance of transgenic lines generated by the facility, and ovary transfers on mouse lines that do not breed well naturally to maintain such lines.

How much does it cost to generate transgenic mice?
Transgenic Mouse Production: The current fee for transgenic mouse production for UTMB investigators is $4000 (for 3 days of injections into [C57BL/6 X C3H/He]F2 embryos) or $5200 (for 4 days of injection into C57BL/6 or FVB/N embryos; $1250 for each additional day requested)[1]. The remainder of the cost is subsidized by the Sealy Center for Cancer Cell Biology, and by departmental contributions. This fee includes microinjection of the DNA construct and care of the offspring of the founders until these mice are 20 days of age. The investigator pays for the per diem mouse cage charges, starting with the day that microinjected eggs are transferred into foster mothers. This cost is currently $0.69/cage/day, and is billed to the investigator directly by ARC.

Incidental costs:
(1) Mice used for mating transgenic founders: C57BL/6 or C3H/He mice: $17/mouse[1].
(2) Special services requested by the investigator while mice are in our care: $28.40/hr[1].
(3) Maintenance of transgenic stocks (after offspring of founder mice are > 20 days of age): $28.40/hr[1].

How effective is the Transgenic Core?
The efficiency of transgenic mouse production is excellent. We have generated an average of 10 founders per construct when we inject into (C57BL/6 X C3H/He)F2 (B6C3F2) embryos. We have produced transgenic mice for over 62 constructs that we have microinjected, with the generation of over 650 individual transgenic founder mice.

How long does it take to establish a transgenic line?
It generally takes about 6-8 months before the investigator can begin experiments on a transgenic line. A detailed time course for the generation of a transgenic line is located at the beginning of this document.

Is consultation available?
We provide advice on all aspects of the development of transgenic mouse models from experimental design to mouse breeding. Please call Jeffrey Ceci to schedule an appointment (Ph. 409-772-2811).

What paperwork must be completed?
The UTMB Animal Care and Use Committee (IACUC) and Biological Safety Committee (use of recombinant DNA) must approve your project. The paperwork for IACUC approval can be obtained from the IACUC web site (see "Links to other sites" on our web page), and the paperwork for Biological Safety Committee approval can be obtained by calling Biological and Chemical Safety (Ext. 21781). **We have a sample IACUC protocol that is available upon request.** In addition, you must also fill out a request for transgenic mouse production. We microinject constructs in the order that we receive this paperwork, along with the construct. If many constructs are received from one investigator, it may be necessary to alternate microinjection of constructs between different investigators.

What is a transgenic mouse?
The genome of a transgenic mouse has a transgene in addition to its normal complement of genes. The transgene is constructed using recombinant DNA technology. The purified DNA is microinjected into the pronucleus of a fertilized 1-cell egg, and the microinjected eggs are then transferred into foster mothers. The transgene integrates randomly into the genome (often as multiple copies in a head-to-tail orientation), and is transmitted as a Mendelian trait. Transgenic founder mice are then identified and bred to produce transgenic lines.

What is involved in making transgenic mice?
The investigator is responsible for designing the transgenic construct and providing purified plasmid DNA. The Core will purify the insert for microinjection. The Core routinely microinjects DNA into fertilized B6C3F2 eggs, and transfers the eggs into foster mothers. Alternatively, we can make transgenic mice in other genetic backgrounds (i.e., C57BL/6, FVB/N), upon request (the investigator is required to pay the additional costs). When the pups are 3 weeks old, the Core applies ear tags and obtains tail biopsies from potential transgenic mice. The investigator isolates genomic DNAs from tail biopsies and identifies the transgenic mice by Southern blot or PCR. The core will breed the transgenic founder mice until offspring are born. When these offspring are 20 days of age, the investigator will be responsible for the care and maintenance of the transgenic lines. We will provide an orientation (if needed) to your laboratory about how to collect tail biopsies and how to maintain knockout lines.

What can I do to maximize a successful transgenic outcome?
Injecting highly purified linear DNA fragments with overhanging ends can optimize the yield of transgenic mice. Removal of as much vector sequence as possible from the

construct is important because prokaryotic sequences can inhibit transgene expression. One method of testing the integrity of the transgenic construct is to determine if the construct can be expressed in cell lines transfected with the construct. If we fail to generate 2 transgenic founder mice after 3 days of injection, we will perform 1 additional day of microinjection free of charge (if we are using B6C3F2 donor embryos- this guarantee does not apply for donor embryos derived from inbred strains). However, we cannot guarantee that the transgene will be transmitted through the germline, or that individual lines will express the transgene.

How many transgenic founder mice will I get?
We have averaged 10 transgenic founder mice per construct when we use donor embryos derived from B6C3F2 mice. The single most important factor that determines the number of transgenic founder mice is the purity of the microinjection DNA.

Can the copy number of the transgene be controlled?
No. Previous work has shown that dilution of microinjected DNA does not affect copy number, but does reduce the overall yield of transgenic mice.

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