Published in final edited form as:

Methods Mol Biol. 2009 ; 590: 335–362. doi:10.1007/978-1-60327-378-7_22.

Transgenic Mouse Technology: Principles and Methods

T. Rajendra Kumar^{1,2,3,4,*}, Melissa Larson^{2,5}, Huizhen Wang^{1,2}, Jeff McDermott⁵, and Illya Bronshteyn⁵

¹Center for Reproductive Sciences, University of Kansas Medical Center, Kansas City, KS 66160

²Departments of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160

³Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160

⁴Institute of Maternal and Fetal Biology, University of Kansas Medical Center, Kansas City, KS 66160

⁵Transgenic and Gene-targeting Institutional Facility, University of Kansas Medical Center, Kansas City, KS 66160

Abstract

Introduction of foreign DNA into the mouse germ line is considered a major technical advancement in the fields of developmental biology and genetics. This technology now referred to as transgenic mouse technology has revolutionized virtually all fields of biology and provided new genetic approaches to model many human diseases in a whole animal context. Several hundreds of transgenic lines with expression of foreign genes specifically targeted to desired organelles/cells/tissues have been characterized. Further, the ability to spatio-temporally inactivate or activate gene expression in vivo using the “Cre-lox” technology has recently emerged as a powerful approach to understand various developmental processes including those relevant to molecular endocrinology. In this chapter, we will discuss the principles of transgenic mouse technology, and describe detailed methodology standardized at our Institute.

*Correspondence: T. Rajendra Kumar, Ph.D., Department of Molecular & Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, Tel: 913-588-0414, tkumar@kumc.edu.

⁶Primer sets should be tested and optimized prior to genotyping. Optimal annealing temperature and MgCl₂ concentration should be determined using genomic DNA (negative control) and genomic DNA spiked with linearized transgene at one copy number per genome (positive control).

⁸Another common problem occurs when embryos lyse after microinjection. A proficient microinjectionist may lyse only 1 – 5% of injected embryos, but the standard rate of lysis post-injection is between 10 and 20%. New injectionist will likely see a much higher rate of lysis (50% or greater). Embryos will lyse if the opening of the injection needle is too great and causes irreparable damage to the membranes. If this is the case, discard the needle and try again with a fresh needle. Similarly, lysis will be high if the rate of DNA flow is too great. This may happen if the pressure is too high on the Femtojet or if the opening of the injection needle is too large. Flow can be ascertained after penetrating the plasma membrane: if movement of the cytoplasm is evident, the flow is too high. Another common source of lysis occurs when withdrawal of the injection needle pulls out the nucleoli of the pronucleus. The tip of the needle is likely sticky, either from embryo debris or large pieces of DNA. It is possible that attempts to remove the material by brushing against the holding pipette may work, but it is advisable to change the needle. The embryos will likely lyse in time, even if they appear intact immediately following injection.

Keywords

Transgene; Mouse Embryo; Pseudopregnancy; Microinjection; Gene Manipulation; Reporter Genes; Integration of Foreign DNA

1. Introduction

Gene manipulation has been the constant pursuit of geneticists since the end of the 19th century. Although initially started as a means to improve and select for the good qualities of species, the potential of gene manipulation was not realized until random mutagenesis screens were devised in bacteriophages and fruit flies in order to score the resultant phenotypes (1). Several advances in gene cloning, chromosomal mapping and DNA sequencing and a wealth of breeding data on various species have heralded a new era of introducing foreign DNA into chromosomes of the host species (2). This technology often known as the transgenic animal technology has become the most popular method of introducing foreign DNA into a host genome. Mice are routinely used for this purpose, because they are relatively inexpensive, easy to maintain and breed and a large amount of data are available with regard to chromosomal mapping and linkage analysis of many mouse genes (2). Moreover, micromanipulation of one-cell mouse embryos is considered technically relatively easy when compared to that in other species. Our group has generated several lines of transgenic mice that phenocopy many human reproductive diseases. In the following sections, we will discuss general principles of transgenic mouse technology and provide detailed methods in later sections.

1.1. Principles

Foreign DNA can be introduced into the mouse genome mainly by three ways. The first method involves DNA delivery by retroviral infection of mouse embryos at different developing stages. Because of many technical problems, this method is not in practice for routine production of transgenic mice (2).

The second method that has been the widely used procedure since its discovery almost 25 years ago, involves the direct microinjection of foreign DNA into the pronuclei of fertilized one-cell mouse embryos. Because the transgene randomly integrates as one or more copies into the mouse genome prior to embryo cleavage, all cells including those of extraembryonic origin will eventually carry the transgene (3, 4). The microinjected embryos are transferred into oviducts of pseudopregnant foster mothers that subsequently produce the transgene carrying founders at varying frequencies. The founders are typically identified by either a Southern blot or genomic PCR assay, often using the proteinase-K-digested tail DNA and eventually be used to establish independent lines that will vary with regard to the transgene integration site as well as in its copy number. This technology became very popular by the pioneering efforts of Ralph Brinster and Richard Palmiter, although has revolutionized virtually every discipline of biology, has a significant association with the field of molecular endocrinology. It stems from the fact that one of the very first strains of transgenic mice created were gigantic as a result of over expression of growth hormone, a key pituitary hormone (5).

The third method exploits the targeted manipulation of mouse embryonic stem (ES) cells at desired loci by introducing loss or gain of function mutations as small as a single base pair change to megabase range chromosomal alterations (6–9). ES cells are derived from the inner cell mass of E3.5 mouse blastocysts. These cells are pluripotent and can contribute to all cell lineages of the embryo proper when injected into recipient blastocysts (6–9). Typically, the donor and recipient blastocysts are obtained from different coat color mice that enable the easy identification of the resulting offspring, called chimeras that display a characteristic patchy distribution of coat colors. The germline transmission of the mutant allele is achieved by breeding the chimeric male mice with normal control female mice. The resulting heterozygous mice are intercrossed to obtain the homozygous mutant mice usually at 25 % frequency, if the mutation is not detrimental to embryo survival and development (6–9). In addition to the above standard gene targeting approach, gene inactivation can also be achieved both spatially and temporally and in a cell-specific conditionally restricted manner (10–12). The reader is referred to excellent reviews and various other sources for a detailed description of the principles and methods of gene targeting strategies in mice (13, 14). In the following sections, we will describe the standard methodology used at our Transgenic and Gene-targeting Institutional Facility (<http://www.kumc.edu/TGIF/transgenic2>) at the Kansas University medical Center.

2. Materials

2.1. Purification of DNA construct

1. Montage DNA Gel Extraction Kit (Millipore, Billerica, MA) or any other suitable DNA gel extraction kit.
2. 1x Modified TAE Buffer: 40mM Tris-Acetate, pH 8.0, 0.1mM Na₂EDTA. Add 1 part 50x modified TAE buffer (Montage DNA Gel Extraction Kit) to 49 parts nuclease-free water.
3. 3M Sodium Acetate, pH 5.5 (Sigma-Aldrich, St. Louis, MO).
4. 70% and 100% molecular biology grade ethanol (Sigma-Aldrich).
5. EmbryoMax Injection Buffer (Millipore).

2.2. Superovulation and setting up mating of donor females

1. Mice of choice (*see* Note 1).

¹The embryo donor strain is dependent upon the desired background strain for the transgenic line, tempered by the strain with which an injector is willing to work. Inbred C57BL/6 embryos are increasingly requested, as this strain has become the gold standard for experimental research. However, C57BL/6 mice and their embryos present a number of difficulties which decrease the efficiency of producing transgenic mice: a great number of ovulated ova are uninjectable; the cytoplasm of the embryo is granular and the pronuclei are small, which make them difficult to inject; a high proportion of the embryos lyse following microinjection; and few embryos develop to term and live-born pups. For these reasons, many transgenic facilities choose to inject C57BL/6 hybrid embryos, eg. C57BL/6 x SJL = B6/SJL, providing hybrid vigor on a partial C57BL/6 background. If C57BL/6 mice are not desired, FVB/N embryos are the easiest to microinject, due to their clear cytoplasm, large pronuclei and high survivability following microinjection. Inbred C57BL/6 mice should be superovulated between three and four weeks of age. Expect as many as 40 – 50 ova per mouse, of which only 20 may be injectable. Hybrid C57BL/6 mice may be superovulated between four and five weeks of age. The number of ova per mouse should average about 30, most of which should be injectable. Inbred FVB/N mice respond well to superovulation between five and six weeks of age. These mice yield fewer ova, about 20 per mouse, but nearly all should be injectable.

2. Pregnant Mare's Serum Gonadotrophin (PMSG) (Sigma-Aldrich G-4877) reconstituted to 50 iu/ml in sterile physiological saline (0.9%) or Dulbecco's Phosphate Buffered Saline (D-PBS). Aliquot into 1.5 ml microcentrifuge tubes and store at -80° . PMSG may also be administered in the form of P.G. 600 (Intervet, Millsboro, DE), a product sold in vials of 400 iu PMSG and 200 iu hCG combined. Reconstitute one vial in 8 ml PBS to achieve 50 iu/ml PMSG.
3. Human Chorionic Gonadotrophin (hCG) (Sigma-Aldrich C-1063) reconstituted to 50 iu/ml. Aliquots may then be frozen at -80° until use.
4. Donor female mice in groups of five or 10 between three and four weeks of age. (See Notes at the end regarding choice of donor strain).

2.3. Production of pseudopregnant recipient females

1. Vasectomized male mice of unimportant genetic status (20 to 30), eg. outbred CD-1 (Charles River, MA) or Swiss Webster (Harlan, IN). Mice may be requested with vasectomy surgery completed prior to shipment. Alternatively, perform vasectomy on 6 week old males.
2. Working solution of 2.5% Avertin anesthetic, prepared from 100% Avertin solution. Prepare stock 100% Avertin by suspending 5 g 2,2,2-Tribromoethanol in 5 ml tert-Amyl alcohol in an amber Boston round glass bottle with an open-closure top (septum screw cap). Allow the suspension to remain overnight at room temperature or heat gently with agitation. Prepare working solution in small batches by diluting 0.25 ml of 100% Avertin solution with 9.75 ml sterile D-PBS (or 0.9% sterile saline). Dissolve overnight or heat gently until the Avertin crystals dissipate, then filter through a 0.22 μ m syringe filter using a 10 cc syringe. Store at 4°C in an amber glass bottle and protect from light. As an alternative, a combination of Ketamine (100 mg/kg body weight) and Xylazine (10 mg/kg body weight) may be administered as an injectable anesthetic. Prepare a cocktail of these two drugs by diluting with PBS: 2 ml Ketamine (50 mg/ml), 0.5 ml Xylazine (20 mg/ml) and 7.5 ml PBS. Store at 4°C .
3. Surgical instruments, including: #5 Swiss jeweler forceps; 3½ " iris scissors; McPherson-Vannas micro iris scissors; clip applicator and clips; and 5-0 absorbable suture with a tapered needle.
4. Recipient female mice (available pool of approximately 50) of unimportant genetic status between the ages of two to five months, eg. outbred CD-1 or Swiss Webster.

2.4. Collection of embryos

1. M2 mouse embryo culture medium buffered with HEPES (MR-015-D, Millipore). This medium is used for handling embryos outside the incubator. Thaw overnight at 4°C prior to use. Medium has a shelf-life of two weeks from thaw date when stored at 4°C .
2. Hyaluronidase for the dispersion of cumulus cells from the surface of the harvested embryos. Prepare by suspending 30 mg of Type IV Hyaluronidase (Sigma-Aldrich

H-4272) in 3 ml sterile D-PBS. Aliquot 100 μ l into microcentrifuge tubes and store at -20°C until use.

3. Bicarbonate buffered mouse embryo culture medium, KSOM (MR-106-D Millipore). This medium is used to culture embryos in an atmosphere with 5% CO_2 . Thaw overnight at 4°C prior to use. Medium has a shelf life of two weeks from thaw date when stored at 4°C .
4. Light mineral oil (M-8410, embryo tested, Sigma-Aldrich).
5. Surgical instruments, including: (4) pair of #5 Swiss jeweler forceps, 3 1/2" iris scissors; and micro iris scissors.
6. Petri dishes, 35 mm.
7. Transfer pipettes are purchased in packs of 20 (#14314 Vitrolife, Englewood, CO). They can also be prepared from 1.5 mm OD x 1.17 mm ID glass transfer capillary (GC150T-10, Harvard Apparatus Part #30-0062, Holliston, MA) using mouth pipetting device (15" aspirator tube assembly, Drummond Scientific 2-000-000, Broomall, PA) (*see* Note 2).
8. CO_2 incubator maintained at 5% CO_2 , 20% air or a tri-gas incubator maintained at 5% CO_2 , 5% O_2 .

2.5. Preparation of DNA construct for microinjection

1. Microinjection buffer (modified TE buffer): 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA (or MR-095-10F, Millipore).

2.6. Pronuclear microinjection of embryos

1. Inverted microscope equipped with either Hoffman Modulation Contrast (HMC) or Differential Interference Contrast (DIC) optics (Nikon Ti-U). The microscope should also be equipped with a stage warmer set at 37°C (Brook Industries, OH) (Figs. 1–4).
2. Plastic dishes for microinjection with HMC or glass depression slides for DIC.
3. Micrometer (Eppendorf Cell Tram Air and/or Oil) for controlling the suction of the holding pipette, and a microinjector or micrometer to allow injection of DNA (Eppendorf Femtojet), as well as manipulators to control the motion of both the holding pipette and injection needle.
4. Glass capillary tools: holding pipettes (VacuTip Eppendorf #9 30001015 or Vitrolife #14318) or prepare from glass capillary (GC100-15, Harvard Apparatus Part #30-0017) (*see* Note 3); injection needle (InJek needle, MicroJek, Kansas City,

²Transfer pipettes are prepared by pulling a capillary tube by hand. Hold a GC150T-10 capillary at both ends; heat the center of the tube over a low flame (either ethanol burner or bunsen burner) until pliable and pull hands apart in a swift and steady motion. The thin neck of the capillary can then be scored with a diamond pen and separated into two pipettes. The tip of the transfer pipette can be polished by holding close to the flame or the heating filament on a microforge, to prevent gouging the bottom of a plastic dish and potentially damaging embryos, especially for users new to manual pipetting. The internal diameter should be approximately 150 μm ; a smaller diameter provides greater control but requires a steadier hand to pick up embryos.

MO) or prepare from 1.0 mm OD x 0.78 mm ID glass capillary with an internal filament (GC100TF-10, Harvard Apparatus Part #30-0038)(*see* Note 4).

5. Incubator maintained at 37°C and 5% CO₂.
6. Stereomicroscope (Nikon SMZ-1000, Japan).
7. 60 mm Petri dish for microinjection with HMC optics.
8. M2 mouse embryo handling medium (Millipore).
9. Light mineral oil (Sigma-Aldrich).

2.7. Embryo transfer into recipient females

1. Stereomicroscope (Nikon SMZ-1000).
2. Working solution of 2.5% Avertin.
3. Animal weighing scale.
4. Mouth pipetting device and glass transfer pipette.
5. 35 mm petri dish.
6. M2 mouse embryo medium (Millipore).
7. Animal clippers with a #50 blade.
8. Alcohol swabs and povidone iodine prep pads.

2.8. Genotyping of pups for transgene positives

1. Dissecting scissors and forceps.
2. Lysis buffer: 50 mM Tris pH 7.6, 100 mM EDTA, 1.0% SDS. Prepare buffer fresh from reagent stock solutions just prior to use.
3. 20.0 mg/ml Proteinase K.
4. Saturated NaCl greater than 6M (should have precipitate on bottom of bottle).
5. 70% and 100% molecular biology grade ethanol.
6. TE buffer: 10 mM Tris-Cl, pH 7.5, 1.0 mM EDTA.
7. Taq polymerase and components: Taq DNA Polymerase (5 U/μL), 10X PCR Buffer (contains 15 mM MgCl₂), dNTP Mixture (2.5 mM each dNTP) (Taq HS DNA Polymerase, Hot Start Version, Takara Bio USA, Madison, WI).

³Holding pipettes are prepared in a manner similar to transfer pipettes. Heat a GC100-15 capillary over a flame and pull apart. Score and separate the two pieces, and place one pipette on the microforge. Polish the tip of the pipette by lowering close to the heating filament, without touching, and heat until the opening of the pipette is almost closed. This will prevent damage to the embryos when immobilizing in the injection dish, and the small opening will prevent the embryos from being drawn into the pipette when suction is applied.

⁴Injection needles are pulled on a mechanical pipette puller (Kopf or Sutter P-97). Load the injection capillary GC100TF-10, taking care not to touch one end of the tube. After pulling, remove the clean half and backload DNA by immersing the back end in the DNA solution. The tip of the needle will fill with DNA by capillary action through the internal filament.

8. DNA primer stocks (Integrated DNA Technologies, Coralville, IA) (*see* Note 5). Reconstitute samples with ddH₂O to a stock concentration of 100 μ M and store at -80°C . Dilute to a working concentration of 10 μ M prior to use and store working solution at -20°C .

3. Methods

3.1. Purification of transgene DNA fragment

1. Purify the undigested plasmid containing the transgene construct by either CsCl gradient or by a membrane column method (e.g., Qiagen Plasmid Maxi/Midi Kit or Promega Wizard Plasmid Purification Kit). The design of typical transgene constructs is illustrated in Fig 5.
2. Restriction digest the vector with appropriate restriction enzymes to completely release and separate the transgene from vector/prokaryotic sequence. The construct must therefore be designed with unique restriction enzyme(s) at each terminus of the transgene (at both junctions with the prokaryotic backbone), permitting isolation by restriction enzyme digest.
3. Electrophorese the products of the restriction digest through a <1.25% agarose gel in 1x Modified TAE buffer.
4. Using a transilluminator, excise the DNA band with a razor and place into the Montage DNA Gel Extraction Device. The volume should be less than 100 μ g.
5. Close lid and spin assembled device for 10 minutes at 5000x g . When finished, discard the Sample Filter Cup and Gel Nebulizer.
6. Determine the volume of your sample. Add 1/10 volume of 3M Sodium Acetate pH 5.5 and 2.5 volumes 100% ethanol. Mix and centrifuge at 15,000x g for 15 minutes. Wash with 70% ethanol, air dry, and resuspend in EmbryoMax Injection Buffer. Concentration should be greater than 2.0 ng/ μ l.

3.2. Superovulation

1. Day 0 is day of embryo microinjection. Begin superovulation on d -3. Thaw desired aliquots of PMSG gently by holding in your hand until all ice has melted. Invert the tube to mix and load a 1 cc insulin syringe. Restrain female mouse and expose abdomen for an intraperitoneal (i.p.) injection. Administer PMSG at 5 iu per female; therefore dose each female 100 μ l or 0.1 cc. Time of administration should be early afternoon, eg. 2:00 pm. After injection, return females to their regular housing.

⁵When designing primers, a unique product must be generated to distinguish the introduced transgene from the host genome. Once the sequence is decided upon, primer selection can be based on traditional rules of thumb (avoid runs over 3 nucleotides; primer length of 18–26 bp; GC content between 40% and 60%; 3' end should be G or C; avoid palindromes and hairpin sequences; avoid complementation between primer pairs; and select melting temperature between 55 to 70 $^{\circ}\text{C}$) or use online primer design programs (Primer3: WWW primer tool, University of Massachusetts Medical School, MA).

2. Administer hCG 46 to 48 hours after PMSG injection (d-1) by i.p. injection at 5 iu per mouse. After injection, pair females with a singly-housed, intact stud males and allow to mate overnight.
3. The next morning, remove females from the males and check for copulation plugs.
4. Also on d-1, prepare embryo culture dishes by placing four 20 μ l drops of KSOM on the bottom of two 35 mm petri dishes. Overlay with light mineral oil and equilibrate overnight at 37°C in a humidified incubator at 5% CO₂ (and 5% O₂ if tri-gas incubator is available).

3.3. Production of Pseudopregnant recipient females

- 1 Assuming outbred stud males are purchased already vasectomized from a commercial source, breeding of recipient females needs to begin at least the day before embryo microinjection is scheduled (d -1).
- 3 If in-house vasectomies are performed, the following procedure can be used.
 1. Anesthetize a six-week old CD-1 male by i.p. injection of 2.5% Avertin at 0.015 ml per gram of body weight. Check that the male is unconscious by toe pinch reflex.
 2. Lay the male on his back and clip the hair of the lower abdomen with a #50 blade. Prep the incision site with alcohol, povidone iodine and alcohol again.
 3. Sterilize instruments by immersing the tips in a bead sterilizer at 250°C. Using forceps, grasp the skin just antral of the penis and make a transverse incision.
 4. Using clean forceps, grasp the body wall and cut transversely just antral of the fat pads that lie beneath the skin. A testicular complex can be exteriorized by grasping the fat pad that lies adjacent to the bladder.
 5. As the testis appears, a long curved tube can be seen. Grasp the vas deferens with a pair of forceps and hold away from the body cavity. Over a burner, flame the tips of a pair of forceps and when red, grasp the tube on both sides of the forceps, effectively removing a loop of the vas deferens while cauterizing the ends.
 6. Return the testis to the body cavity and repeat on the other side.
 7. Stitch body wall with 5-0 absorbable suture with a tapered needle. Close skin with two wound clips. Allow the male to recover in a cage on a warmed surface until ambulatory.
 8. Remove wound clips at one week. Check that vasectomy is complete by test-mating males two weeks after surgery by pairing naturally-cycling adult outbred female mice with vasectomized males at 1–2 females per male.

9. Euthanize the female the next morning and look for sperm in the reproductive tract, or mate and wait 10 days to determine if pregnant. If no sperm or pregnancies, males can be used to generate pseudopregnant recipients.
2. Pair naturally-cycling adult outbred female mice with vasectomized males at 1–2 females per male. Add the females to the male's cage, rather than adding the male to the females' cage. Establish at least 15 mating pairs.
3. The next morning, remove females from the males and check for copulation plugs. The morning of plugging is considered d 0.5, these females are suitable for oviduct transfer.

3.4. Collection of embryos

1. Prepare 35 mm dishes of medium by transferring in a sterile manner 3 ml of M2 into three dishes. To one dish, thaw and add the aliquot of hyaluronidase that was previously prepared.
2. Sterilize surgical instruments by immersing the tips in a bead sterilizer for 15 seconds. Allow to cool down while keeping the tips clean.
3. Euthanize plugged female mice by cervical dislocation or CO₂ inhalation. Prepare females by laying on their back on an absorbent surface and wetting the abdomen with 70% ethanol (to reduce the distribution of cut hair).
4. Grasp the skin of the lower abdomen of each mouse and make a single transverse cut with the iris scissors. The skin may then be pulled back by grasping the incision site and the tail and pulling in opposite directions.
5. Now grasp the body wall. Again make a transverse cut across the lower abdomen, and use the scissors to increase the cuts to either side of the body. Fold body wall back on top of the thorax.
6. Using clean forceps, displace the intestines to the outside of the body cavity and locate the kidneys along each side of the spine. The ovaries will lie rostral to each kidney, with the oviducts and uterine horns attached. Pick up an ovary with one hand and stretching gently, use the micro iris scissors to cut the connective membrane between the ovary and oviduct. Now grasp the space between the oviduct and uterine horn and cut this connection in a similar manner. Place the excised oviduct into a dish of M2. Repeat this procedure for both oviducts of all donor mice.
7. Transfer the excised oviducts to the dish of M2 and hyaluronidase. Under a stereo microscope, use clean forceps to immobilize one oviduct on the bottom of the dish. Locate the ampulla (the swollen section of the oviduct, which will appear extended and clear) and tear open with the tip of the other forceps. The cumulus mass containing the embryos will “pour” out of the oviduct, usually with no further manipulation required. Remove the empty oviduct from the dish and repeat the process for all of the remaining oviducts.

8. After all oviducts have been removed from the dish, attach a transfer pipette to a mouth aspirator assembly. Pick up embryos that have fallen out of the cumulus mass as the cells disperse in the hyaluronidase.
9. Wash embryos in the third dish of M2, wash in KSOM, transfer to a culture drop of KSOM and place in incubator until microinjection.

3.5. Preparation of transgene DNA for Microinjection

1. Just prior to microinjection, prepare 50 μ l of a working dilution of the transgene DNA at 1 ng/ μ l in microinjection buffer.
2. Spin small constructs at high speed for 10 min in order to pellet any particulate debris in the suspension. Carefully remove the top half of the suspension and transfer to a clean microcentrifuge tube. Maintain at 4°C for use that day.

3.7. Microinjection

1. Prepare a microinjection dish by placing a 75–100 μ l drop of M2 in the center of a 60 mm petri dish. Overlay the drop with light mineral oil. Place the injection dish on the inverted scope and turn on stage warmer to 37°C.
2. Assemble holding pipette and place on manipulator.
3. Pull a microinjection needle and carefully place the blunt end into the DNA solution. Allow the tip to load by capillary action. Load needle into holder and turn on Femtojet. The microinjector will require some time to establish pressure within the system, but a needle must be loaded prior to turning on the Femtojet. Similarly, any time that a needle requires changing, the Femtojet must be paused.
4. Lower both the holding pipette and the injection needle into the microinjection drop, first manually and then adjust with the vertical control of the manipulators. Both instruments should be positioned horizontally across the field of view.
5. Remove embryos from the incubator and transfer a small quantity (20 if learning, 50 or more when proficient) to a dish of M2. (Return the KSOM dish with remaining embryos to the incubator.) Pick embryos up in a small volume of M2 and on low magnification on the inverted microscope, deposit in the microinjection drop just south of the glass capillary tools.
6. While on low magnification, dial back on the holding pipette to establish suction and attempt to pick up an embryo (Fig 6). Adjust vertical position of the pipette as necessary, to be as close as possible to the level of the embryo without the pipette dragging on the bottom of the dish.
7. Switch to high magnification. Position the embryo so that the pronuclei are not obscured by the polar bodies. Also minimize the real estate of the embryo that must be traversed prior to reaching a pronucleus with the injection needle. For obvious reasons, it is prudent to aim for the larger of the two pronuclei.
8. Once the embryo is positioned, bring the pronuclear membrane into focus by adjusting the fine focus on the microscope. Next, bring the injection needle close to

the edge of the embryo. Focus the very tip of the needle by raising and/or lowering the manipulator, without adjusting the focus on the microscope. In this way, the needle will be on the same plane of focus as the pronucleus.

9. Advance the needle into the embryo and puncture the pronuclear membrane (Fig 7). Inject the DNA solution until the pronucleus visibly swells, then withdraw the needle swiftly but gently (*see* Notes 7–⁹). Injection needles may need to be changed with some frequency during injection, as they may become clogged or sticky.
10. Move the injected embryo to the top of the microinjection dish and repeat with the next embryo. When all embryos in the dish have been injected, pick up the embryos, wash through KSOM, transfer back into a culture drop of KSOM and return to the incubator for keeping until embryo transfer surgery. Remove the next group of embryos for microinjection and repeat the process until all are injected.

3.7. Transfer of embryos to recipient females

1. Embryo transfer surgery requires area for animal preparation, embryo handling, surgery and recovery. Begin by weighing the recipient female (identified earlier by copulation plug). Avertin (2.5%) is administered i.p. at 0.015 ml per gram of body weight (BW). Alternatively, Ketamine/Xylazine is administered i.p. at 0.01 ml/g BW. Allow the anesthetic several minutes to take effect.
2. Remove injected embryos from the incubator and transfer to a dish of M2. Sort embryos into groups of 25 after removing all embryos that lysed following microinjection.
3. Remove the hair from the back of the mouse by clipping with a #50 blade. Wipe the clipped area with an alcohol prep pad to remove hair and scrub with a povidone prep pad. Follow with another alcohol wipe and move the mouse to the surgery area.
4. Sterilize all instruments by immersing the tips in a bead sterilizer at 250°C. With forceps, pick up the skin lying immediately over (dorsal) the area of the kidney on one side of the mouse and make a small incision with iris scissors.
5. With clean forceps, pick up the body wall beneath the incision and make a small opening. The ovary will lie rostral to the kidney and can be identified by the large fat pad attached to it. Grasp the fat pad, exteriorize the reproductive tract and lay over the back of the mouse by clamping the fat pad with a bulldog Serrafin clamp.

⁷If DNA fails to cause the pronucleus to swell, check that the microinjector (Femtojet) is in inject mode. Test the needle by attempting to inject an embryo. If the pronucleus does not swell, the tip of the injection needle may need to be broken. If so, focus on the holding pipette and adjust the manipulator height until the tip of the needle is also in focus. Insert the very tip of the needle into the opening of the holding pipette and gently brush the needle against the side of the holding pipette. This should break the tip, creating an opening through which DNA can pass. If it appears that the needle is broken open sufficiently and still no DNA comes out, it may be that material is covering the opening of the needle. In this case, discard the needle and prepare and load a new needle.

⁹Very large DNA constructs, such as bacterial and yeast artificial chromosomes (BACs and YACs), require special handling to ensure that the construct remains intact before and during microinjection. But it should also lead to a greater number of transgenic mice incorporating the entire construct versus fragments of the BAC or YAC. Inject the freshly prepared BAC/YAC at a concentration of 0.5 – 1.0 ng/μl, using wide bore tip. It will be necessary to break the tip to create a larger opening (than for small DNA constructs). Avoid vigorous pipetting as this will likely lead to a DNA shearing and higher incidence of lysis.

6. Load embryos into the transfer pipette in as small a volume as possible. Load M2 medium, an air bubble, medium, another air bubble and the embryos in single file. Aspirate an additional air bubble and a small amount of medium after the embryos at the tip of the transfer pipette. The loaded aspiration assembly can then be suspended over the microscope eyepiece while the oviduct is exposed.
7. Move the mouse to the stage of the microscope and focus on the oviduct. The infundibulum will lie dorsal and slightly below the rest of the oviduct (at the junction with the ovary). Identify the infundibulum and using clean forceps, tear the bursa immediately above the opening of the oviduct, taking care to avoid blood vessels.
8. Pick up the aspiration assembly and position the transfer pipette at the opening of the infundibulum. Insert the pipette and grasp the outside of the oviduct over the pipette with forceps in the opposite hand. Expel the contents of the pipette until two air bubbles appear in the ampulla (those before and after the embryos in the transfer pipette).
9. Remove the clamp and return the tract to the body cavity. It is not necessary to suture the body wall. Close the skin with a wound clip. Place the recipient female in a new cage and allow to recover on a warmed surface until ambulatory. Repeat for any additional embryos and recipients. If there are insufficient recipients on d 0, embryo transfers may be performed at the two-cell stage the following day into 0.5 d recipients.

3.8. Genotyping and identification of founders

- 1 Pups will be born between d19 and 21. Wean pups at three weeks of age and collect tail biopsies. Scruff pups, identify by ear punch or other means and cut off a small piece of the tail (0.5 cm). Place the tail biopsy in a microcentrifuge tube and mark with the identification number of the mouse. Place on ice while other samples are collected. Rinse dissecting scissors and forceps with 70% ethanol between each sample. Store all tail biopsies at -20°C until ready to process.
- 4 If the DNA extracted from the tail is just for PCR analysis, modern DNA isolation kits will work (Sigma's REDEExtract-N-Amp Tissue PCR Kit, Sigma, St. Louis, MO) and have been proven to be cheap, quick and reliable. If further analysis of the DNA is needed (eg. Southern blotting), most kits are not suitable as they will shear the genomic DNA to a size less than 10Kb. The following is a traditional proteinase K digestion that will yield genomic DNA to a size greater than 40Kb.
- 5 Lyse tail in 500 μl Lysis Buffer (50 mM Tris pH7.6, 100 mM EDTA, 1.0% SDS). Add 20 μl 20 mg/ml Proteinase K and incubate at 55°C overnight.
- 6 Add 250 μl saturated 6M NaCl.
- 7 Shake tube approximately 500 times. Do not vortex to prevent shearing of genomic DNA.

- 8 Chill on ice for 10 min. Centrifuge at 7000 rpm for 10 min.
- 9 Aspirate 650 µl of supernatant and transfer to new microcentrifuge tube. Add 2 volumes of 100% ethanol, invert 10–20 times. Spool DNA with a glass rod (capillary tube with one end sealed) and rinse with 70% ethanol. Resuspend in 50 µl TE buffer.
- 10 Determine the quality of the DNA prep by spectrophotometer (good quality = 260/280 reading between 1.6 and 2.0). If quality of the prep is poor (spectrophotometer 260/280 reading > 2.0 or < 1.6), a phenol:chloroform extraction can be performed to decrease the DNA contaminants. The DNA must be precipitated again after phenol:chloroform extraction. Store resuspended DNA at –20°C.
- 11 Thaw all PCR reagents, templates, and controls on ice. Taq polymerase should be kept at –20°C.
- 12 Mix following in PCR tube for each reaction. A Master Mix of buffer, dNTP's, primers, Taq and water can be prepared and aliquoted into PCR tubes, to which the sample DNA is then added for each tail sample.

Template (25–200 ng/µl)	x µl
10 x Buffer (with MgCl ₂)	5.0 µl
2.5 mM dNTP	4.0 µl
10 µM primer-Forward	2.0 µl
10 µM primer-Reverse	2.0 µl
Taq (1.25 U/µl)	0.25 µl

Add sterile ddH₂O to a final volume of 50 µl (see Note 10).

- 12 Place into thermocycler and program the PCR reaction as follows:

Step	Temperature °C	Time
1	94	2 min
2	94	15 sec
3	*	15 sec
4	72	**
5	Goto step 2	30 times
6	72	5 min
7	10	--

* Annealing temperature may vary from primer set to primer set, optimizing may be necessary. °C = [4(G + C) + 2(A + T)] – 5, for only primers < 20 bp.

** For extension times use 1 minute per 1 kB expected fragment size.

- 13 Run PCR products on an agarose gel and visualize on a transilluminator (Fig 8).

¹⁰The concentration of MgCl₂ can be optimized by using 10x PCR buffer without MgCl₂ and increasing the final concentration from 0.5 to 2.5mM by increments of 0.5mM MgCl₂ until a strong band can be visualized when the PCR products are run on an agarose gel.

3.9. Applications of transgenic technology

Transgenic technology offers limitless opportunities to determine in vivo gene functions in numerous ways. In general, the transgenes are expressed in a tissue/cell-specific manner using either homologous (i.e. mouse) or heterologous (for example, rat, cow, sheep, human, pig, etc.) gene regulatory sequences (3, 4). Mouse genes or cDNAs appropriately marked with random oligos or engineered with heterologous downstream polyadenylation DNA sequences are also used (3, 4). The effects of overexpression of transgenes on organ development and/or physiology are then monitored. In some instances, ectopic expression of the transgene is also achieved by purposefully directing its expression to tissues/cells different from those in which the corresponding mouse gene is normally expressed (3, 4).

In several instances, data obtained with cell transfection studies on mapping the regulatory regions of genes that confer tissue/cell specific expression most often are not very well correlated or difficult to interpret, compared to the corresponding in vivo scenario. In such cases, transgenic approach is often used to identify, map and define the minimal regulatory elements of a given promoter that dictate tissue/cell specific expression and hormonal regulation. This is usually achieved by first engineering a series of deletion constructs from a larger piece of the gene that is known to confer tissue/cell specific expression. Subsequently, the truncated transgenes are microinjected to produce transgenic mice and their expression in the selected cell type is monitored at the RNA and/or protein level as an endpoint (15). Similar strategies have also been used in which a known transcription factor-binding site is mutated on a given promoter driving the expression of a transgene and its functional consequence tested in vivo (16–20). In case, the promoter elements of a given gene are already identified and characterized, these can be used to direct the expression of useful reporters that can be quantitatively assayed. The commonly used reporters for quantifying the promoter activity include lacZ from *E. coli*, chloramphenicol acetyl transferase and the firefly luciferase (2).

Developmental expression of many genes that have important endocrine function can be tracked using lineage marking and cell fate mapping. Depending on specificity and expressivity of the gene regulatory sequences and the earliest time at which these are activated as early as during embryogenesis, expression of either lacZ, alkaline phosphatase or various fluorescent reporters (for example, GFP, CFP, YFP or dsRed) can be targeted to specific cell types (2). Tissues/cells are harvested in such cases, and the activity of lacZ (formation of a blue product), alkaline phosphatase (formation of blue or red product) or the visualization of distinct colors under ultraviolet illumination is monitored starting from embryonic stages (2). Thus, tracking such “reporter-tagged” cells through distinct developmental stages will provide a novel way to study the lineage specification and differentiation of desired cell types. Furthermore, cell-cell interactions during organogenesis can sometimes be visualized on a short-term basis in a Petri dish by live cell imaging using confocal microscopy. Because cells expressing fluorescent reporters can be sorted by fluorescence activated cell sorting, these transgenic mice will also provide novel resources to purify desired cell types from a tissue consisting of heterogeneous populations of various other cells (21–23). These can be further used for gene/protein expression profiling under normal physiological or pathological conditions.

Transgenic approaches that identify cell/specific regulatory elements have also been useful for selectively ablating cells at desired times and study the consequences of the loss of hormones secreted from these cells (2). This has been achieved by expressing either diphtheria toxin (17), or herpes simplex virus thymidine kinase (24), or viral-specific ion channels (25). In the latter two cases, either an appropriate substrate (gancyclovir) or an ionophore (calcium or sodium channel activator or blocker) is used to produce either a cell-toxic product or changes in ion flux that affect hormone secretion, respectively. These approaches have been used, for example, to study the consequences of ablation of gonadotropes on gonadal development and reproduction (24). More recently, ablation of Sertoli cells has been achieved to study the consequences on germ cell development and function that consequently impact male reproduction (26).

Transgenic strategies permit immortalization of rare cell types that are often difficult to obtain in large numbers and good purity for routine cell transfection analyses (27–32). This is usually achieved by targeted expression of viral oncogenes to immortalize desired cell types in vivo (33). Moreover, novel cell lines are derived from these tumors and established as useful in vitro tools for various studies. Since many cell types within the endocrine organs are post-mitotic, this approach has been particularly very useful for immortalizing these endocrine cell types and establishing novel cell lines (27–32). Many of these cell lines have been used to investigate specific signal transduction pathways, and transcriptional regulation (27–32). In some cases, these tumor-prone mouse models also phenocopy known human cancers and thus have tremendous potential to understand the pathobiology of the human disease (29). Furthermore, these models can also be a useful resource for identifying novel cancer biomarkers.

In many cases, the expression of a given transgene is dependent on the promoter that drives its expression, and in some cases, the site of its integration and copy number will also dictate the expression of the transgene. In addition, some promoters also exhibit high basal activity in many tissues (34, 35). This feature has been exploited to ectopically express various hormones in multiple tissues at high basal levels and the consequences analyzed (36–38). Some of these promoters are also inducible, for example, metallothionein-1 promoter is induced by heavy metals such as zinc and cadmium (34, 35).

A strategy to temporally induce transgene expression has also been developed. In this approach, the desired protein encoding cDNA or gene is engineered downstream of a conditionally activated “gene switch” (39, 40). This “gene switch” consists of DNA sequences that often encode a bacterial repressor protein and an operator region that is linked to the gene/cDNA of interest. The repressor binds to the adjacent operator DNA sequence that is fused in tandem with the gene/cDNA of interest and keeps it inactive. This entire “gene switch” cassette along with the gene/cDNA of interest is engineered under the regulation of a tissue/cell specific promoter. When a drug that binds the repressor and prevents it from binding to the operator region, is administered to transgenic mice, it allows the transcription of the desired gene/cDNA at specific times. Based on this principle transgenic mice have been developed using tet^{on} (41), ecdysone (39, 42), RU486 (43), tamoxifen (44) gene switches as inducible gene expression models.

Many transgenic lines of mice have also been successfully used in gene targeting experiments to genetically rescue knockout mutant mice, spatio-temporally inactivate genes by using the Cre-lox approach, cell fate mapping and gene/protein expression profiling.

3.10. Resources

In the above sections we have described a summary of various principles of transgenic mouse technology. We encourage the reader to browse through excellent mouse genome resources available as public databases (Table 1). The Jackson Labs in Barr Harbor, ME and the mouse genome informatics are great resources for all the mouse genome based information. This is tightly linked to the National Center for Biotechnology Information (NCBI) that provides various mouse genomics tools. Similarly, ENSEMBL website maintained by the The Sanger Institute in Cambridge, UK has numerous resources related to genomes of mouse, human and other species. A number of Institution-based web sites also provide transgenic and other mouse genome resources (Table 2). There are several ready-to-use practical manuals and reference books that describe various aspects of transgenic and knockout mouse technologies (2, 14, 45). Finally, species other than mouse have also been successfully used for transgenesis including chicken (46, 47), cow (48–50), fish (salmon and zebra fish) (51–54), frog (55, 56), goat (57–59), sheep (50, 60), pig (61, 62), rabbit (63–65), rat and monkey (67, 68). However, several methodological refinements are ongoing in several laboratories to routinely achieve transgenesis in species other than rodents.

Acknowledgments

T.R.K. acknowledges funding support from NIH (NCRR Center for Biomedical Research Excellence Program Grant RR024214, HD04394, HD056082 and K-INBRE P20 RR016475) and The Hall Family Foundation, Kansas City, MO. H.W. is the recipient of a postdoctoral fellowship from the Biomedical Research Training Program at the Kansas University Medical Center.

References

1. Griffiths, AJF.; Wessler, SR.; Lewontin, RC.; Carroll, SB. Introduction to Genetic Analysis. W.H. Freeman & Co; New York: 2008.
2. Nagy, A.; Gertsenstein, M.; Vintersten, K.; Behringer, R. Manipulating the mouse embryo: A laboratory manual. 3. Cold Spring Harbor Laboratory; New York: 2003.
3. Palmiter RD, Brinster RL. Germ-line transformation of mice. *Annu Rev Genet.* 1986; 20:465–499. [PubMed: 3545063]
4. Brinster RL, Palmiter RD. Introduction of genes into the germ line of animals. *Harvey Lectures.* 1984; 80:1–38. [PubMed: 6400653]
5. Palmiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC, Evans RM. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature.* 1982; 300:611–615. [PubMed: 6958982]
6. Bradley A, van der Weyden L. Mouse: Chromosome Engineering for Modeling Human Disease. *Annu Rev Genomics Human Genetics.* 2006; 7:247–276. [PubMed: 16824018]
7. Capecchi MR. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet.* 2005; 6:507–512. [PubMed: 15931173]
8. Evans M. Embryonic stem cells: a perspective. *Novartis Found Symp.* 2005; 265:98–103. discussion 103–106. [PubMed: 16050252]
9. Koller BH, Smithies O. Altering genes in animals by gene targeting. *Annu Rev Immunol.* 1992; 10:705–730. [PubMed: 1591000]

10. Le Y, Sauer B. Conditional gene knockout using Cre recombinase. *Mol Biotechnol.* 2001; 17:269–275. [PubMed: 11434315]
11. Sauer B, Henderson N. Cre-stimulated recombination at loxP-containing DNA sequences placed into the mammalian genome. *Nucleic Acids Res.* 1989; 17:147–161. [PubMed: 2783482]
12. Porret A, Merillat AM, Guichard S, Beermann F, Hummler E. Tissue-specific transgenic and knockout mice. *Methods Mol Biol.* 2006; 337:185–205. [PubMed: 16929948]
13. Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M, Collins FS, et al. The knockout mouse project. *Nat Genet.* 2004; 36:921–924. [PubMed: 15340423]
14. Joyner, AL. *Gene Targeting: A practical approach.* 2. Oxford University Press; New York: 2001.
15. Kumar TR, Schuff KG, Nusser KD, Low MJ. Gonadotroph-specific expression of the human follicle stimulating hormone beta gene in transgenic mice. *Mol Cell Endocrinol.* 2006; 247:103–115. [PubMed: 16414183]
16. Hamernik DL, Keri RA, Clay CM, Clay JN, Sherman GB, Sawyer HR, et al. Gonadotrope- and thyrotrope-specific expression of the human and bovine glycoprotein hormone alpha-subunit genes is regulated by distinct cis-acting elements. *Mol Endocrinol.* 1992; 6:1745–1755. [PubMed: 1280329]
17. Kendall SK, Saunders TL, Jin L, Lloyd RV, Glode LM, Nett TM, et al. Targeted ablation of pituitary gonadotropes in transgenic mice. *Mol Endocrinol.* 1991; 5:2025–2036. [PubMed: 1665205]
18. Keri RA, Bachmann DJ, Behrooz A, Herr BD, Ameduri RK, Quirk CC, et al. An NF-Y binding site is important for basal, but not gonadotropin-releasing hormone-stimulated, expression of the luteinizing hormone beta subunit gene. *J Biol Chem.* 2000; 275:13082–13088. [PubMed: 10777613]
19. Keri RA, Nilson JH. A steroidogenic factor-1 binding site is required for activity of the luteinizing hormone beta subunit promoter in gonadotropes of transgenic mice. *J Biol Chem.* 1996; 271:10782–10785. [PubMed: 8631889]
20. Quirk CC, Lozada KL, Keri RA, Nilson JH. A single Pitx1 binding site is essential for activity of the LHbeta promoter in transgenic mice. *Mol Endocrinol.* 2001; 15:734–746. [PubMed: 11328855]
21. Balthasar N, Mery PF, Magoulas CB, Mathers KE, Martin A, Mollard P, et al. Growth hormone-releasing hormone (GHRH) neurons in GHRH-enhanced green fluorescent protein transgenic mice: a ventral hypothalamic network. *Endocrinology.* 2003; 144:2728–2740. [PubMed: 12746337]
22. Bonnefont X, Lacampagne A, Sanchez-Hormigo A, Fino E, Creff A, Mathieu MN, et al. Revealing the large-scale network organization of growth hormone-secreting cells. *Proc Natl Acad Sci (U S A).* 2005; 102:16880–16885. [PubMed: 16272219]
23. Magoulas C, McGuinness L, Balthasar N, Carmignac DF, Sesay AK, Mathers KE, et al. A secreted fluorescent reporter targeted to pituitary growth hormone cells in transgenic mice. *Endocrinology.* 2000; 141:4681–4689. [PubMed: 11108283]
24. Markkula M, Kananen K, Klemi P, Huhtaniemi I. Pituitary and ovarian expression of the endogenous follicle-stimulating hormone (FSH) subunit genes and an FSH beta-subunit promoter-driven herpes simplex virus thymidine kinase gene in transgenic mice; specific partial ablation of FSH-producing cells by antiherpes treatment. *J Endocrinol.* 1996; 150:265–273. [PubMed: 8869593]
25. Le Tissier PR, Carmignac DF, Lilley S, Sesay AK, Phelps CJ, Houston P, et al. Hypothalamic growth hormone-releasing hormone (GHRH) deficiency: targeted ablation of GHRH neurons in mice using a viral ion channel transgene. *Mol Endocrinol.* 2005; 19:1251–1262. [PubMed: 15661833]
26. Ahtiainen M, Toppari J, Poutanen M, Huhtaniemi I. Indirect Sertoli cell-mediated ablation of germ cells in mice expressing the inhibin-alpha promoter/herpes simplex virus thymidine kinase transgene. *Biol Reprod.* 2004; 71:1545–1550. [PubMed: 15240419]
27. Alarid ET, Holley S, Hayakawa M, Mellon PL. Discrete stages of anterior pituitary differentiation recapitulated in immortalized cell lines. *Mol Cell Endocrinol.* 1998; 140:25–30. [PubMed: 9722164]

28. Alarid ET, Windle JJ, Whyte DB, Mellon PL. immortalization of pituitary cells at discrete stages of development by directed oncogenesis in transgenic mice. *Development*. 1996; 122:3319–3329. [PubMed: 8898243]
29. Kumar TR, Graham KE, Asa SL, Low MJ. Simian virus 40 T antigen-induced gonadotroph adenomas: a model of human null cell adenomas. *Endocrinology*. 1998; 139:3342–3351. [PubMed: 9645710]
30. Pernasetti F, Spady TJ, Hall SB, Rosenberg SB, Givens ML, Anderson S, et al. Pituitary tumorigenesis targeted by the ovine follicle-stimulating hormone beta-subunit gene regulatory region in transgenic mice. *Mol Cell Endocrinol*. 2003; 203:169–183. [PubMed: 12782413]
31. Thomas P, Mellon PL, Turgeon J, Waring DW. The Lbeta-T2 clonal gonadotrope: a model for single cell studies of endocrine cell secretion. *Endocrinology*. 1996; 137:2979–2989. [PubMed: 8770922]
32. Windle JJ, Weiner RI, Mellon PL. Cell lines of the pituitary gonadotrope lineage derived by targeted oncogenesis in transgenic mice. *Mol Endocrinol*. 1990; 4:597–603. [PubMed: 1704103]
33. Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature*. 1985; 315:115–122. [PubMed: 2986015]
34. Palmiter RD. Molecular biology of metallothionein gene expression. *Experientia Suppl*. 1987; 52:63–80. [PubMed: 2959555]
35. Palmiter RD, Norstedt G, Gelinis RE, Hammer RE, Brinster RL. Metallothionein-human GH fusion genes stimulate growth of mice. *Science*. 1983; 222:809–814. [PubMed: 6356363]
36. Guo Q, Kumar TR, Woodruff TK, Hadsell LA, DeMayo FJ, Matzuk MM. Overexpression of mouse follistatin causes reproductive defects in transgenic mice. *Mol Endocrinol*. 1998; 12:96–106. [PubMed: 9440814]
37. Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, Byrne MC, et al. Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. *Mol Endocrinol*. 1999; 13:851–865. [PubMed: 10379885]
38. Matzuk MM, DeMayo FJ, Hadsell LA, Kumar TR. Overexpression of human chorionic gonadotropin causes multiple reproductive defects in transgenic mice. *Biol Reprod*. 2003; 69:338–346. [PubMed: 12672665]
39. Ryding AD, Sharp MG, Mullins JJ. Conditional transgenic technologies. *J Endocrinol*. 2001; 171:1–14. [PubMed: 11572785]
40. Schnutgen F, Doerflinger N, Calleja C, Wendling O, Chambon P, Ghyselinck NB. A directional strategy for monitoring Cre-mediated recombination at the cellular level in the mouse. *Nat Biotechnol*. 2003; 21:562–565. [PubMed: 12665802]
41. Zhu Z, Zheng T, Lee CG, Homer RJ, Elias JA. Tetracycline-controlled transcriptional regulation systems: advances and application in transgenic animal modeling. *Semin Cell Dev Biol*. 2002; 13:121–128. [PubMed: 12127145]
42. Karzenowski D, Potter DW, Padidam M. Inducible control of transgene expression with ecdysone receptor: gene switches with high sensitivity, robust expression, and reduced size. *Biotechniques*. 2005; 39:191–192. 194, 196. [PubMed: 16116792]
43. Pierson TM, Wang Y, DeMayo FJ, Matzuk MM, Tsai SY, O'Malley BW. Regulable expression of inhibin A in wild-type and inhibin alpha null mice. *Mol Endocrinol*. 2000; 14:1075–1085. [PubMed: 10894156]
44. Albanese C, Hulit J, Sakamaki T, Pestell RG. Recent advances in inducible expression in transgenic mice. *Semin Cell Dev Biol*. 2002; 13:129–141. [PubMed: 12240598]
45. Marten, H.; Hofker, JVD.; Deursen, JV. *Transgenic Mouse: Methods and Portocols (Methods in Molecular Biology)*. Vol. 209. Humana Press; Totowa, N.J: 2003.
46. Han JY. Germ cells and transgenesis in chickens. *Comp Immunol Microbiol Infect Dis*. 2008 Epub ahead of print.
47. Houdebine LM. Production of pharmaceutical proteins by transgenic animals. *Comp Immunol Microbiol Infect Dis*. 2008 Epub ahead of print.
48. Laible G, Wells DN. Transgenic cattle applications: the transition from promise to proof. *Biotechnol Genet Eng Rev*. 2006; 22:125–150. [PubMed: 18476329]

49. Melo EO, Canavessi AM, Franco MM, Rumpf R. Animal transgenesis: state of the art and applications. *J Appl Genet.* 2007; 48:47–61. [PubMed: 17272861]
50. Robl JM, Wang Z, Kasinathan P, Kuroiwa Y. Transgenic animal production and animal biotechnology. *Theriogenology.* 2007; 67:127–133. [PubMed: 17070901]
51. Halpern ME, Rhee J, Goll MG, Akitake CM, Parsons M, Leach SD. Gal4/UAS transgenic tools and their application to zebrafish. *Zebrafish.* 2008; 5:97–110. [PubMed: 18554173]
52. Higashijima S. Transgenic zebrafish expressing fluorescent proteins in central nervous system neurons. *Dev Growth Differ.* 2008; 50:407–413. [PubMed: 18445066]
53. Houdebine LM, Chourrout D. Transgenesis in fish. *Experientia.* 1991; 47:891–897. [PubMed: 1915772]
54. Male R, Lorens JB, Nerland AH, Slinde E. Biotechnology in aquaculture, with special reference to transgenic salmon. *Biotechnol Genet Eng Rev.* 1993; 11:31–56. [PubMed: 7840846]
55. Chesneau A, Sachs LM, Chai N, Chen Y, Du Pasquier L, Loeber J, et al. Transgenesis procedures in *Xenopus*. *Biol Cell.* 2008; 100:503–521. [PubMed: 18699776]
56. Ishibashi S, Kroll KL, Amaya E. A method for generating transgenic frog embryos. *Methods Mol Biol.* 2008; 461:447–466. [PubMed: 19030816]
57. Niemann H, Kues W, Carnwath JW. Transgenic farm animals: present and future. *Rev Sci Tech.* 2005; 24:285–298. [PubMed: 16110896]
58. Niemann H, Kues WA. Application of transgenesis in livestock for agriculture and biomedicine. *Anim Reprod Sci.* 2003; 79:291–317. [PubMed: 14643109]
59. Van Cott KE, Velander WH. Transgenic animals as drug factories: a new source of recombinant protein therapeutics. *Expert Opin Investig Drugs.* 1998; 7:1683–1690.
60. Campbell KH, Fisher P, Chen WC, Choi I, Kelly RD, Lee JH, et al. Somatic cell nuclear transfer: Past, present and future perspectives. *Theriogenology.* 2007; 68(Suppl 1):S214–S231. [PubMed: 17610946]
61. Dobrinski I. Germ cell transplantation in pigs--advances and applications. *Soc Reprod Fertil Suppl.* 2006; 62:331–339. [PubMed: 16866328]
62. Prather RS, Sutovsky P, Green JA. Nuclear remodeling and reprogramming in transgenic pig production. *Exp Biol Med (Maywood).* 2004; 229:1120–1126. [PubMed: 15564438]
63. Chrenek P, Makarevich AV, Pivko J, Massanyi P, Lukac N. Characteristics of rabbit transgenic mammary gland expressing recombinant human factor VIII. *Anat Histol Embryol.* 2009; 38:85–88. [PubMed: 19143684]
64. Kondo M, Sakai T, Komeima K, Kurimoto Y, Ueno S, Nishizawa Y, et al. Generation of a transgenic rabbit model of retinal degeneration. *Invest Ophthalmol Vis Sci.* 2008 Epub ahead of print.
65. Li S, Guo Y, Shi J, Yin C, Xing F, Xu L, et al. Transgene expression of enhanced green fluorescent protein in cloned rabbits generated from in vitro-transfected adult fibroblasts. *Transgenic Res.* 2008 Epub ahead of print.
66. Murphy D. Production of transgenic rodents by the microinjection of cloned DNA into fertilized one-celled eggs. *Methods Mol Biol.* 2008; 461:71–109. [PubMed: 19030792]
67. Chan AW, Chong KY, Martinovich C, Simerly C, Schatten G. Transgenic monkeys produced by retroviral gene transfer into mature oocytes. *Science.* 2001; 291:309–312. [PubMed: 11209082]
68. Chan AW, Luetjens CM, Dominko T, Ramalho-Santos J, Simerly CR, Hewitson L, et al. TransgeniCSI reviewed: foreign DNA transmission by intracytoplasmic sperm injection in rhesus monkey. *Mol Reprod Dev.* 2000; 56:325–328. [PubMed: 10824995]
69. Kumar TR, Low MJ, Matzuk MM. Genetic rescue of follicle-stimulating hormone beta-deficient mice. *Endocrinology.* 1998; 139:3289–3295. [PubMed: 9645705]
70. Huang HJ, Sebastian J, Strahl BD, Wu JC, Miller WL. Transcriptional regulation of the ovine follicle-stimulating hormone-beta gene by activin and gonadotropin-releasing hormone (GnRH): involvement of two proximal activator protein-1 sites for GnRH stimulation. *Endocrinology.* 2001; 142:2267–2274. [PubMed: 11356672]
71. Huang HJ, Sebastian J, Strahl BD, Wu JC, Miller WL. The promoter for the ovine follicle-stimulating hormone-beta gene (FSHbeta) confers FSHbeta-like expression on luciferase in

transgenic mice: regulatory studies in vivo and in vitro. *Endocrinology*. 2001; 142:2260–2266. [PubMed: 11356671]

Figure 1.
Photograph of a micromanipulator

Figure 2.
Injection platform of a micromanipulator

Figure 3.
Injection needle pullers

Figure 4.
Pipette polisher

Figure 5.

Design of transgenes. Some representative examples of transgenes that we and other groups have used are shown. The top construct contains 10kb of *HFSHB* sequences (~4 kb of 5' promoter sequences, 2 kb of 3' downstream sequences and all three exons shown as black boxes with numbers below) and was designed to test the gonadotrope-specific and hormonal regulation in pituitaries of transgenic mice (15). Middle panel contains a 1.8 kb mouse metallothionein-1 (MT1) promoter that drives the expression of *HFSHB* sequences. This transgene was designed to achieve *FSHB* expression to multiple ectopic tissues (37, 69). The bottom panel shows a 4.7 kb sheep *FSHB* promoter-luciferase transgene (*oFSHB-Luc*) that was designed to test the regulation of sheep *FSHB* promoter by GnRH, activins and various other transcription factors (70, 71).

Figure 6.
A mouse embryo held with a holding pipette is ready for microinjection of DNA.

Figure 7.
A mouse embryo photographed just after microinjection of transgene DNA into the male pronucleus.

Figure 8.

Example of a typical genomic PCR gel depicting amplified products from a transgene (Brca1) and an internal control (GAPDH) gene. The left most lane in top and bottom panels contains 100 bp DNA size markers. Tg: transgene fragment amplified in founder mice. GAPDH: Primers for glyceraldehydes-3-phosphate dehydrogenase encoding gene (GAPDH) used as an internal control for checking the presence of DNA in each lane. +: Amplified products from positive control plasmids containing Tg (top panel) or GAPDH (lower panel) DNA sequences.

Table 1

List of web links to some useful mouse genome resources

International Gene Trap Consortium (http://www.genetrap.org)
Baygenomics (http://baygenomics.ucsf.edu)
The Jackson Laboratory (General site) (http://jax.org)
Federation of International Mouse Resources (http://www.fimre.org)
European Mouse Mutant Archive (http://www.emma.rm.cnr.it)
Ensembl Mouse (http://www.ensembl.org/Mus_musculus/index.html)
Mouse Genome Informatics (http://www.informatics.jax.org)
Trans-NIH Mouse Initiative (http://nih.gov/science/models/mouse/resources/index.html)
Lexicon Genetics (http://www.lexicon-genetics.com/index.php)
Baylor College of Medicine (ENU project) (http://www.mouse-genome.bcm.tmc.edu)
Cornell University (ENU project) (http://www.vertebrategenomics.cornell.edu)
The Jackson Laboratory (Infertility website) (http://jaxmice.jax.org/library/notes/496c.html)
The Sloan-Kettering Mouse Project (http://mouse.ski.mskcc.org)

Table 2

Useful Institutional websites for transgenic core facilities

Institute	Website
1. Johns Hopkins Univ. Transgenic Core Laboratory	www.hopkinsmedicine.org/core/home.htm
2. U Pennsylvania Transgenic & Chimeric Mouse Facility	www.med.upenn.edu/genetics/core-facs/tcmf
3. Stanford University Transgenic Mouse Research Facility	med.stanford.edu/transgenic
4. University of Michigan Transgenic Animal Model Core	www.med.umich.edu/tamc
5. U Pittsburgh Transgenic& Chimeric Mouse Facility	www.genetics.pitt.edu/services/labpage.html?whichlab=tcmf
6. Duke University Transgenic Mouse Facility	www.cancer.duke.edu/tmf/about