

Guidelines for Production of Transgenic Mice by Pronuclear Injection

Introduction:

The injection of a DNA solution into the pronuclei of fertilized eggs is the most common method for making transgenic animals. Injection is done at the stage of development when mammalian ova have two pronuclei, one from each gamete, which will later fuse to form the diploid nucleus. Mice are particularly suitable for this procedure because it is easy to collect large numbers of fertilized eggs from a relatively small number of animals (around 5 females are used per injection day). Also, the pronuclei of mouse eggs are easy to visualize using Differential Interference Contrast (DIC) optics.

The egg donors are given two hormone injections, spaced 46-48 hours apart, to cause them to release more than the usual number of eggs – a process known as superovulation. They are then mated with fertile males and the eggs are harvested the next morning.

After injection, the eggs are transferred to the oviducts of pseudopregnant foster mothers, generated by mating females with vasectomized males so the females do not produce any fertilized embryos of their own. The offspring resulting from injected eggs may or may not carry the transgene. The mice that do carry the transgene are called founders.

Pups are genotyped using DNA extracted from a tissue biopsy (usually a small piece of tissue cut from the tip of the tail).

The TMF at the University of Oregon have produced in 2006 more than 80 transgenic founders coming from more than 15 different transgenes reaching as much as 35% of founders from the born first generation, with an average of 10%.

Ordering:

An ordering form (provided by TMF) is filled up by the investigator and given to the TMF along with the DNA material. The form has to be entirely completed and the instructions followed carefully in order to the order to be processed.

One set of injections includes 300 eggs injected, which would give birth to up to 50 pups with up to 10 transgenic lines, depending of the quality of DNA). At least 30 pups born is the minimum guaranteed. The number of transgenics obtained can vary from 0 to #10 (or more) and is dependent of the quality of the DNA (there is no guarantees there). We guaranty 500 eggs injected or 250 eggs re-implanted or 50 pups born whichever comes first.

In case of failure, a second round of injection will be performed for free (subject to condition: see performance guarantees)

In addition the investigator must provide evidence of approval from his institution's Animal Care and Use Committee for his animal use protocol

Service Description:

The service includes:

- purchase of egg donors
- superovulation of egg donors and mating with stud males maintained by the TMF
- creation of pseudopregnant foster mothers by mating outbred ICR females with vasectomized males and checking for vaginal plugs the morning of injection
- harvesting of eggs from euthanized donors
- transfer of all eggs that survive the injection process into the oviducts of pseudopregnant foster mothers
- monitoring of foster mothers before and after birth of pups
- marking pups in each litter by ear-punch, sexing, tail-cutting and weaning at about 3 weeks of age
- Genotyping (if not waived by the client)
- transfer of tail biopsies to the client for genotyping if requested by the client
- weaning, identification, and transfer or shipment of transgenic pups to the client

Extra services can be asked to be done by the TMF:

- Helping in the design of the transgene
- Purification of the fragment to inject

Turnaround Times:

The minimum time required to produce transgenic founders that can be transferred to the client is about 8 weeks, once we have received a construct ready for microinjection and the necessary paperwork. This includes about 2 weeks to order and prepare the egg donors, 3 weeks gestation time, and 3 weeks from birth to weaning.

The turnaround time can be much longer, either because of previously scheduled orders or because of a failure to produce founders from the first injection. Delays may also be caused by technical difficulties in identifying transgenic founders, which is generally the responsibility of the client.

When you want to schedule an injection you can either:

a) Wait until the construct is finalized and ready before asking for an injection spot. In that case you do not have to worry about any possible waste of mice. In another hand you might have to expect some delay as the injections are processed in the order they are received.

b) Take a risk with scheduling in advance your injection by projecting your expected date to be ready. If you are not ready on time you can:

- Cancel the injection at least 3 weeks in advance.
- If we can find another project to replace the one that cannot be done, the mice purchased can be transferred to the new investigator. Nevertheless, if the mice were already in the facility, you would have paid for their housing until their transfer.
- If no other project can replace yours, the mice will stay at the responsibility (and charge) of the PI who they were ordered originally for.

In order to minimize turnaround times, we recommend optimizing and testing your genotyping protocol well in advance of pups being born. For PCR assays, test the specificity and sensitivity by diluting your transgene into genomic DNA from mouse tails (available from the TMF), down to a concentration equivalent to a single-copy gene. For a transgene that is 3 kb in length, this would be equivalent to 0.1 picogram of transgene in 100 nanograms of genomic DNA.

Performance guarantees:

It is the client's responsibility to make sure the DNA construct has been purified correctly and its concentration determined accurately. Inaccuracies in the concentration of as little as 2-fold can have significant effects on the production of transgenic founders. The gel picture serves as documentation that the DNA has been purified correctly and its concentration is accurate.

We guaranty 500 eggs injected or 250 eggs reimplanted or 50 pups born whichever comes first. On a regular basis, we prefer to have the TMF performing the genotyping on the first generation pups. Having the TMF performing the genotyping of the first generation provides the assurance of quality.

If no transgenic line is obtained at the first try, a second round of injection will be performed for free. If the investigator decides to waive this genotyping option and decides to perform the genotyping himself, he also waives the second free injection in case of a poor result. It is the responsibility of the investigator to provide genotyping material (primers, probe), information (PCR protocol) and the appropriate controls to the TMF.

Breeding Transgenic Founders:

Transgenes are integrated at random into the genome. Thus, each founder will have a different site of integration. The number of copies of the transgene possessed by each founder may also be different. For these reasons, each founder should be treated as a separate line and bred independently of other founders with the same transgene.

Offspring must be obtained from each founder to test for transmission and expression of the transgene. Due to position effects and different copy numbers, each founder line can have a different level of expression. However, we cannot guarantee that any expression of the transgene will be obtained.

The transgene will not necessarily be transmitted in a Mendelian fashion by a given founder. Founders can be mosaic for the transgene, if integration occurs after the first cell division. Mosaicism can result in a frequency of inheritance of less than 50% in the first generation offspring. In some founders, the transgene may integrate into more than one locus, resulting in a frequency of inheritance of more than 50%. In this case, the expression levels among the first generation offspring may vary, depending on which integration site they inherit.

A more uncommon problem is loss of the transgene altogether. This may be caused by meiotic recombination, as in a double-crossover event.

Founders being from the hybrid CB6F1 strain, the client must decide whether to maintain a mixed background or to backcross to an inbred line. In this case it will be necessary to backcross to the desired background for at least 6 generations to achieve a true congenic.

Mice that have the transgene on one chromosome are termed “hemizygous” because they do not have a corresponding allele on the other chromosome. It is possible to produce homozygous transgenics, and these may have a higher level of expression than the corresponding hemizygous mice, but distinguishing homozygotes from hemizygotes can be difficult. Some kind of quantitative genotyping assay must be used (e.g., quantitative PCR or Southern blotting). Alternatively, suspected homozygotes can be crossed to wildtype mice and all offspring tested to see if they are hemizygous.

We do not recommend however to create transgenic homozygotes as it often leads to unexpected phenotypes. Due to the random integration of the transgene the hemizygote integration site's phenotype might be dormant but is revealed in its homozygotic form.