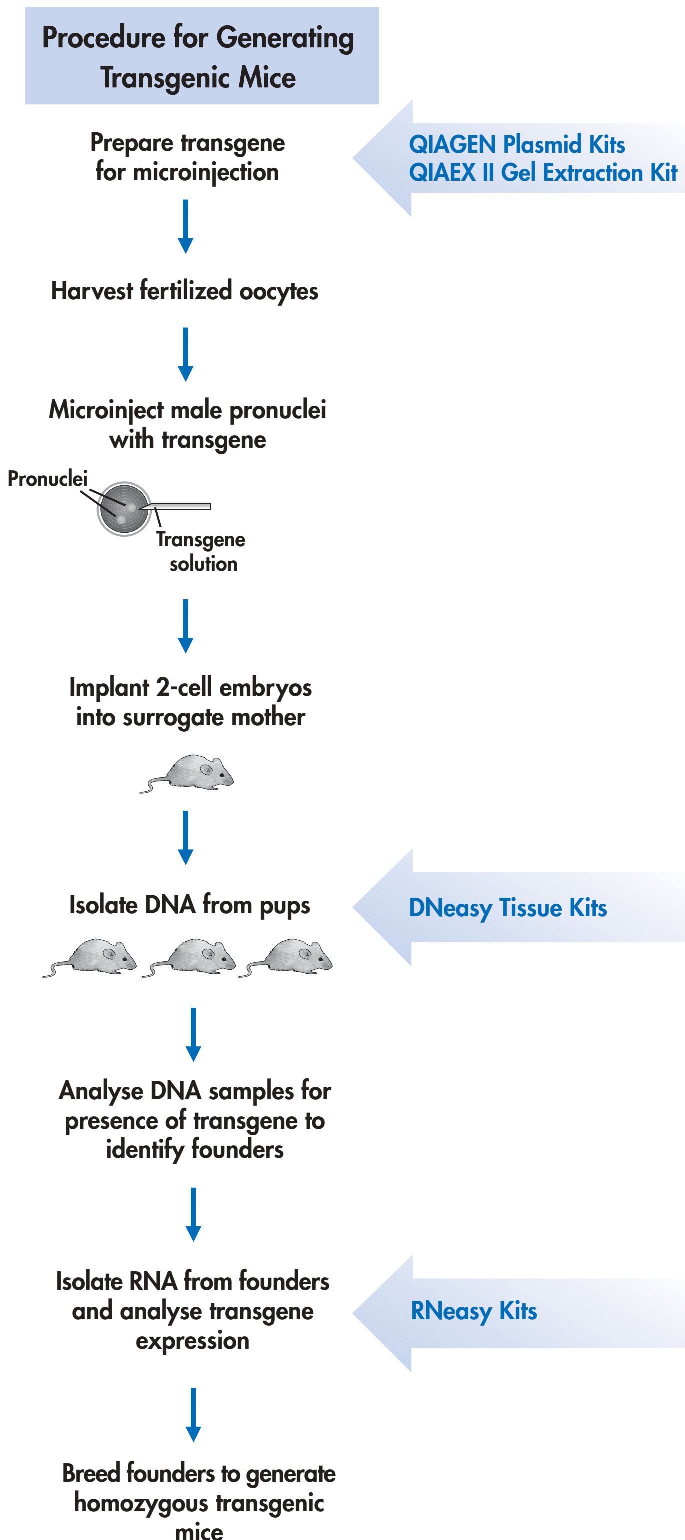
Important considerations for generating transgenic mice

The development of technology to manipulate the genome of animals has revolutionized biology, biomedical research, and biotechnology. The ability to add a specific gene to, or remove a specific gene from, a complex organism allows precise characterization of gene function, and allows generation of animal models for studying specific diseases and developmental processes. The animals can also be used for testing pharmacological molecules or gene therapy protocols.

This article gives a short overview of the procedure as well as important considerations for generating transgenic mice, in which an extra gene (transgene) is randomly inserted into the genome. The discussion applies equally to generation of other transgenic animals.

Generation of transgenic mice

Pronuclear DNA microinjection is the most commonly used method to generate transgenic mice (see flowchart). Fertilized oocytes are removed from the oviduct of a mouse, and the male pronucleus is microinjected with a solution containing the transgene. The injected eggs are cultured in vivo until the pronuclei have fused and the zygote has developed into a 2-cell embryo. The embryos are then transplanted into a surrogate mother, and pups are born 19–21 days later. Pups that developed from a zygote that successfully integrated the microinjected DNA will have the transgene in every cell of their body. These heterozygous animals (called founders) can then be bred to obtain homozygous mice.



Important considerations

Transgene preparation

Preparation of the transgene used for microinjection is critical for successful generation of transgenic animals. In general, transgenes derived from genomic DNA are expressed at higher levels than those derived from cDNA (1). The vector construct must contain the necessary promoter elements for expression of the transgene in the host cells. ▶

References

1. *Transgenic Core, Boston University School of Medicine (www.bu.edu/index.html)*
2. *NICHD Transgenic Mouse Development Facility, University of Alabama (main.uab.edu/sys/images/pdf/NICHD.pdf)*
3. *Transgenic Mouse Core Facility, University of Virginia Health System (www.med.virginia.edu/medicine/inter-dis/transgenic-mouse/DNAinj.html)*
4. *Blumberg, H. et al. (2001) Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell* ***104****, 9.*
5. *Merscher, S. et al. (2000) TBX1 is responsible for cardiovascular defects in Velo-cardio-facial/DiGeorge syndrome. Cell* ***104****, 619.*
6. *Moeller, M.J., Kovari, I.A., and Holzman, L.B. (2000) Evaluation of a new tool for exploring podocyte biology: mouse Nphs 1 5' flanking region drives LacZ expression in podocytes. J. Am. Soc. Nephrol.* ***11****, 2306.*
7. *Transgenic animals (www.acs.ucalgary.ca/~browder/transgenic.html)*

Linearization of the vector and removal of unnecessary vector sequences through the use of appropriate restriction enzymes usually improves integration success and leads to higher expression rates (1, 2).

Vector DNA must be highly pure for successful generation of transgenic mice (1). Use of degraded DNA will result in lower numbers of transgenic pups, or none at all. Contaminants present in the DNA can be harmful to the embryo and prevent incorporation of the vector into the genome and/or expression of the transgene, and particulate matter can clog the microinjection pipettes. Several transgenic core facilities recommend the use of QIAGEN® Plasmid Kits for isolation of ultrapure plasmid DNA and the QIAEX® II Gel Extraction Kit for purification of DNA fragments from agarose gels following restriction digestion (e.g., 1, 2, 3).

Identification of founders

Approximately 10–20% of pups can be expected to be transgenic. The litter must therefore be screened to identify founder animals. Both Southern-blot and PCR analysis can be used for screening. Southern-blot analysis is typically preferred for identifying founder animals (1) and allows determination of the number of integration sites, transgene copy number, and transgene integrity. PCR analysis is typically used for monitoring the breeding process once the founder(s) has been identified.

DNA for screening is usually isolated from tail samples and should be highly pure to avoid false positive and false negative results (1). Since only a small percentage of the pups is expected to be transgenic, the time required for screening should be minimized in order to keep animal housing costs low. DNeasy® Tissue Kits have been successfully used for isolation of DNA from mouse tails for screening in transgenic studies (4, 5, 6).

Analysis of transgene expression

Transgene expression can be influenced by the DNA surrounding the insertion site (7) and therefore must be thoroughly characterized before using animals in experimental systems. Transgene expression can be analyzed by northern-blot or RT-PCR analysis.

To avoid false negative results, RNA should be intact and highly pure. RNeasy® Kits have been successfully used for isolation of RNA from mouse tissue for RT-PCR analysis of transgene expression (4).

> “We tested three different methods for isolation of genomic DNA from mouse tail samples. All samples were collected at the same time and processed according to the manufacturer's specifications. The DNeasy 96 Tissue Kit from QIAGEN yielded DNA of excellent quality, while DNA isolated using a home-made precipitation method and a kit from another supplier showed signs of degradation. I would recommend the DNeasy 96 Tissue Kit for ease of use, DNA yield, and DNA quality.”
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> Jim Busby
> Research Associate
> Amgen, Inc., USA

Summary

- Vector DNA purity is a critical factor for successful generation of transgenic mice. Use of DNA purified with QIAGEN kits is recommended by several transgenic core facilities.
- Timely identification of founders requires high-purity DNA to avoid false-positive and false-negative results. DNeasy Tissue Kits provide a fast and convenient method for isolating DNA from mouse tails for use in PCR and Southern-blot analysis, and are highly suited for high-throughput applications.
- Analysis of transgene expression requires intact, pure RNA. RNeasy Kits allow fast and easy isolation of RNA that is suitable for all downstream applications, including northern-blot, slot-blot, and RT-PCR analysis.

A discussion of knock-out mice will appear in a future issue of *QIAGEN News*. ■

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Ordering Information

Product	Contents	Cat. No.
EndoFree® Plasmid Kits — for purification of endotoxin-free ultrapure plasmid DNA		
EndoFree Plasmid Maxi Kit (10)*	10 QIAGEN-tip 500, 10 QIAfilter™ Maxi Cartridges, Buffers	12362
QIAEX II Gel Extraction Kits — for purification of DNA following agarose gel analysis		
QIAEX II Gel Extraction Kit (150)†	For up to 150 extractions: QIAEX II Suspension, Buffers	20021
DNeasy Tissue Kits — for isolation of genomic DNA from animal tissues and cells, yeast, or bacteria		
DNeasy Tissue Kit (50)†	50 DNeasy Spin Columns, Reagents and Buffers, Collection Tubes (2 ml)	69504
DNeasy 96 Tissue Kits‡ — for high-throughput DNA isolation from rodent tails and animal tissues		
DNeasy 96 Tissue Kit (4)†	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Reagents and Buffers, Collection Microtubes (1.2 ml), Collection Microtube Caps, 96-Well-Plate Registers	69581
RNeasy Kits — for isolation of total RNA from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (50)*†	50 RNeasy Mini Spin Columns, RNase-free Reagents and Buffers, Collection Tubes (2 ml)	74104

"QIAGEN broadens its spectrum of detection reagents — Penta·His™ antibody conjugates" (see page 3).

Product	Contents	Cat. No.
Penta·His Alexa Fluor® 488 Conjugate	125 µl Penta·His Alexa Fluor 488 Conjugate	35310
Penta·His Alexa Fluor 532 Conjugate	125 µl Penta·His Alexa Fluor 532 Conjugate	35330
Penta·His Alexa Fluor 555 Conjugate	125 µl Penta·His Alexa Fluor 555 Conjugate	35350
Penta·His Alexa Fluor 647 Conjugate	125 µl Penta·His Alexa Fluor 647 Conjugate	35370
Penta·His Biotin Conjugate	125 µl Penta·His Biotin Conjugate	34440
Streptavidin–R-PE	250 µl Streptavidin–R-phycoerythrin Conjugate	922721

"Significantly higher yields from automated protein purification procedures" (see page 12).

Product	Contents	Cat. No.
Ni-NTA Superflow™ 96 BioRobot® Kit (24)	For 24 x 96 6xHis-tagged protein preps: 24 QIAfilter 96 Plates, 24 TurboFilter 96 Plates, 3 x 100 ml Ni-NTA Superflow	969263
Ni-NTA Superflow (100 ml)§	100 ml nickel-charged resin (max. pressure: 140 psi)	30430

** Different kit formats available; please inquire*
† Larger kit sizes available; please inquire
‡ Requires use of the QIAGEN 96-Well-Plate Centrifuge System
§ Additional Ni-NTA Superflow resin required when using 200 µl per well, as described in the article (page 12).