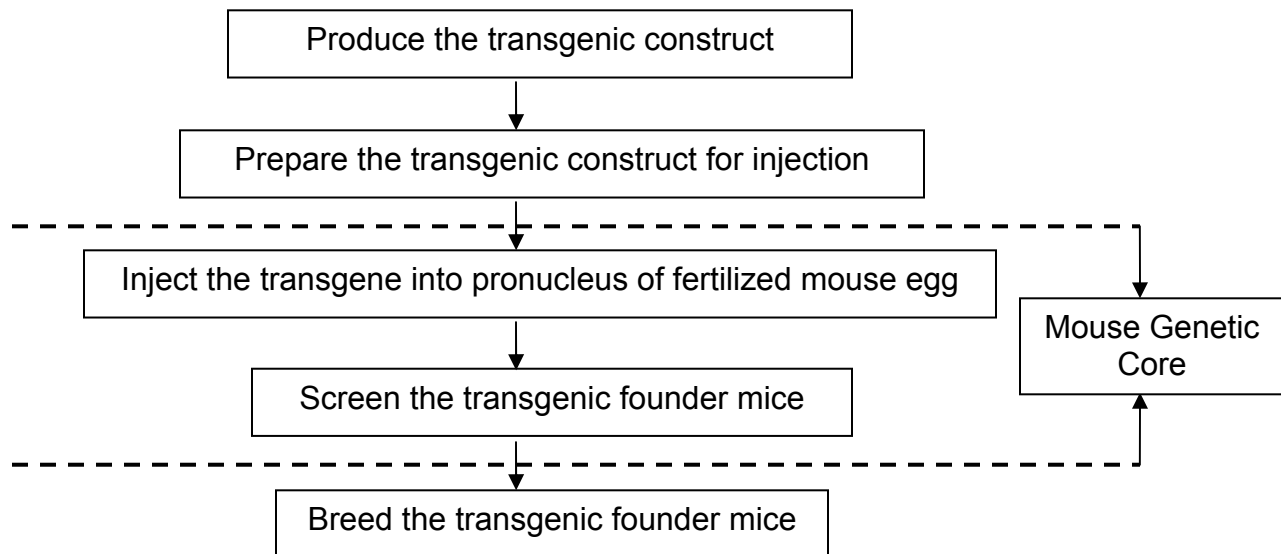


## Production of Transgenic Mice

Transgenic animals are produced by injecting a DNA construct directly into the pronucleus of a newly fertilized mouse egg. In some eggs, the DNA will integrate into the mouse chromosomal DNA before cell division, and will be present in all cells in the mouse including the germ line.

Overview:



### **Produce the Transgenic Construct:**

Clone the cDNA of interest into the expression shuttle vectors, which contain both bacterial amplification and mammalian expression components.

Sequence the transgenic construct.

#### **General considerations**

\*The cDNA of interest is inserted into the multiple cloning sites of expression vectors. The multiple cloning sites are downstream of the eukaryotic promoter sequence, and upstream of poly A signal.

\*The expression shuttle vectors also contain the important prokaryotic sequences for the amplification of the transgenic construct in bacteria.

\*A cloning scheme should be designed with a clear understanding of all the elements and a strategy to free the transgene from vector sequences once constructed. In most cases, this requires checking by a second person (eg; Teresa or Dan) to catch potential problems.

## Paperwork preparation before submission

**Form 1:** (If applicable to Diabetes, check with TCL or DK)  
DRTC TRANSGENIC CORE FACILITY  
REQUEST FOR SERVICE

[www.medicine.wustl.edu/~drtc/cores/transgenic/](http://www.medicine.wustl.edu/~drtc/cores/transgenic/)

Burton Wice, director

\*Please see attachment.

### **Form 2:**

#### **Mouse Genetics Core**

#### **Transgenic Construct Submission Form**

\*Please see attachment. It is a PDF file, could be downloaded from Mouse Genetics Core: [http://mgc.wustl.edu/request\\_forms/](http://mgc.wustl.edu/request_forms/)

\*\*\*It takes a week for DRTC approval, so it's better to start the paperwork process early.

## Prepare the Transgenic Construct for Injection

The transgene and mammalian expression component will be freed from vector sequence with the restriction digestion, and separated by agarose gel electrophoresis. The recovered transgenic fragment will be purified using QIAEX II kit from Qiagen. The DNA concentration will be accurately determined with a fluorometer.

## Free transgenic sequence from shuttle vector with appropriate restriction enzyme digestion

**Considerations:** Save all the mammalian expression components (eukaryotic promoter, cDNA of interest, and polyA signal), and get rid of the bacterial components (plasmid replication sites, antibiotic resistant gene sequence and its promoter) as much as possible. This requires **complete** digestion in most cases.

### **Determine the restriction digestion strategy:**

\*To pick the appropriate restriction enzyme digestion sites, it is better to run software (such as Vector NTI from Invitrogen) to survey the whole transgenic

construct, and make sure there are no unlisted restriction digestion sites within the transgenic sequence for injection.

***Digest the transgenic construct:***

\*Digest enough transgenic construct to yield at least 2 ug of DNA for injection after the purification (typically, each round of gel purification yields 25-50%, and the minimal amount of DNA requested by the core is 500 ng). Thus, if you have a 3260bp transgene in a 5260 bp plasmid, the transgene is 62% of the total DNA. If the plasmid is 3.2ug/ul –  $3.2 \times 0.62 = 1.98$  ug of transgene. Therefore you would digest 1 ul of plasmid which contains about 2ug of transgene.

\*Digest the transgenic construct at 37°C for 2 hour (avoid the extensive digestion of DNA due to the potential star activity of the restriction enzyme).

***Separate the transgenic sequence using agarose gel electrophoresis in TAE or TBE buffer:***

\*After running the gel, visualize the fragment under **long wave** UV light with minimum time of exposure. Ensure good separation of transgenic sequence from the rest of the shuttle vector.

\* For fragments larger than 10Kb, electroelution before QIAEX may improve yield (Consult with Ted Simon in the core about this issue).

**Purify the DNA fragment using QIAEX II kit**

Note: This kit is recommended over the Qiaquick gel extraction kit

\*Exactly follow the instructions of the QIAEX II kit as written below but elute the DNA using 10mM Tris, pH 8.0. Repeat the elution, and combine the two eluants.

**\*\*\*\*\*QIAEX II Agarose Gel Extraction Protocol\*\*\*\*\***

This protocol is designed for the extraction of 40-bp to 50-kb DNA fragments from 0.3–2% standard or low-melt agarose gels in TAE or TBE buffers.

**Notes:** • The yellow color of Buffer QX1 indicates a pH  $\leq 7.5$ .

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- A heating block or water bath at 50°C is required.
- 3M sodium acetate, pH 5.0, may be necessary.
- All centrifugation steps are at maximum speed ( $\geq 10,000 \times g$ ,  $\sim 13,000$  rpm) in a conventional, table-top microcentrifuge.
- For DNA fragments larger than 10 kb, mix by gently flicking the tube to avoid shearing the DNA. Do not vortex the tube.

**1. Excise the DNA band from the agarose gel with a clean, sharp scalpel.**

Minimize the size of the gel slice by removing excess agarose. Use a 1.5-ml microfuge tube for processing up to 250 mg agarose.

**2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QX1 to 1 volume of gel for DNA fragments 100 bp – 4 kb; otherwise, follow the table below.**

For example, add 300 µl of Buffer QX1 to each 100 mg of gel.

DNA fragments <100 bp	Add 6 volumes of Buffer QX1
DNA fragments >4 kb	Add 3 volumes of Buffer QX1 plus 2 volumes of H <sub>2</sub> O
>2% or Metaphor agarose gels	Add 6 volumes of Buffer QX1

**3. Resuspend QIAEX II by vortexing for 30 sec. Add QIAEX II to the sample according to the table below and mix.**

≤2 µg DNA	Add 10 µl of QIAEX II
2–10 µg DNA	Add 30 µl of QIAEX II
Each additional 10 µg DNA	Add additional 30 µl of QIAEX II

**4. Incubate at 50°C for 10 min to solubilize the agarose and bind the DNA. Mix by vortexing\* every 2 min to keep QIAEX II in suspension. Check that the color of the mixture is yellow.**

If the color of the mixture is orange or purple, add 10 µl 3M sodium acetate, pH 5.0, and mix. The color should turn to yellow. The incubation should then be continued for an additional 5 min at least.

The adsorption of DNA to QIAEX II particles is only efficient at pH ≤7.5. Buffer QX1 now contains a pH indicator which is yellow at pH ≤7.5, and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

**5. Centrifuge the sample for 30 sec and carefully remove supernatant with a pipet.**

**6. Wash the pellet with 500 µl of Buffer QX1.**

Resuspend the pellet by vortexing\*. Centrifuge the sample for 30 sec and remove all traces of supernatant with a pipet. This wash step removes residual agarose contaminants.

**7. Wash the pellet twice with 500 µl of Buffer PE.**

Resuspend the pellet by vortexing\*. Centrifuge the sample for 30 sec and carefully remove all traces of supernatant with a pipet. These washing steps remove residual salt contaminants.

**8. Air-dry the pellet for 10–15 min or until the pellet becomes white.**

If 30 µl of QIAEX II suspension is used, air-dry the pellet for approximately 30 min.

Do not vacuum dry, as this may cause overdrying. Overdrying the QIAEX II pellet may result in decreased elution efficiency.

**9. To elute DNA, add 20 µl of 10 mM Tris-Cl, pH 8.5 or H<sub>2</sub>O and resuspend the pellet by vortexing\*. Incubate according to the table below.**

DNA fragments ≤4 kb	Incubate at room temp. for 5 min
DNA fragments 4–10 kb	Incubate at 50°C for 5 min
DNA fragments >10 kb	Incubate at 50°C for 10 min

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) but the EDTA may inhibit subsequent enzymatic reactions.

**10. Centrifuge for 30 sec. Carefully pipet the supernatant into a clean tube.**  
The supernatant now contains the purified DNA.

**11. Optional: repeat steps 9 and 10 and combine the eluates.**

A second elution step will increase the yield by approximately 10–15%

*\* Vortexing can cause shearing of large DNA fragments. For fragments larger than 10 kb, resuspend the pellet by inverting and flicking the tube.*

**\*\*\*\*\*End of QIAEX II protocol\*\*\*\*\***

12. Precipitate the DNA with 1/10 volume 3M sodium acetate, pH=5.2, and 2.5 volumes absolute ethanol.

13. Chill overnight at –20° or for 15 minutes in dry ice/ethanol.

14. Pellet the DNA by centrifugation at 12000 x g (full speed in a microfuge) for 15 minutes.

15. Remove the supernatant, and add an equal volume of cold 70% ethanol, and vortex.

16. Spin the sample again for one minute, and remove the supernatant.

17. Air dry the pellet.

18. Add 20 µL of transgene buffer (10mM Tris, pH=7.4, 0.1mM EDTA) and vortex. Allow the DNA to solubilize for at least 15 minutes with occasional vortexing.

19. Determine the concentration of the DNA with a fluorometer:

Contact Dr. Ted Simon for the availability of fluorometer in his laboratory.

20. Run 200ng of the construct on an agarose gel with appropriate size markers and visualize with ethidium bromide staining. A single, sharp band of the appropriate size should be evident. Attach an original picture of the gel to the "Mouse Genetics Core Transgenic Construct Submission Form".

21. The transgenic construct should be provided in a tube labeled with the construct name and DNA concentration. The construct name **should be short and not contain unusual or Greek characters, subscripts or superscripts.**

21. Store the construct at -20 °C until submission.

### **Mouse Genotyping Preparation**

\*\*\*PCR primers are required for all new transgenic constructs or requests for genotyping service. See details on their website:

[http://mgc.wustl.edu/protocols/pcr\\_genotyping.html](http://mgc.wustl.edu/protocols/pcr_genotyping.html)

The primers for the Mouse Genetic Core should be 30 nucleotides in length, and produce a PCR product between 100 and 500 nucleotides.

Two sets of primers are required. First set of primers is designed to detect the specific transgenic construct, and the second set of primers is used as the internal control, such as FABPi200 (194 bp PCR product), or FABPi500 (466 bp PCR product), depending on the size of the PCR product for your transgenic construct, so the internal control will not interfere with the specific transgenic amplification during dual-primer PCR.

\*Please visit Genetic Core website for more detailed information:

[http://mgc.wustl.edu/protocols/pcr\\_primer\\_set.html](http://mgc.wustl.edu/protocols/pcr_primer_set.html)

\*Test the transgenic construct primer sets before submission. You should be able to visualize the specific PCR bands using the transgenic construct (0.01 pg).

In some cases, the core will already have primers that can be used for your construct. Check with them first, or check the web, for availability of primers.

### **Paperwork Preparation after Submission**

\*File your transgenic construct using **Kelly Lab Plasmid Form** with the map and the sequencing results, and place a copy in the lab binder "MAMMALIAN EXPRESSION VECTORS FOR TRANSGENES"

\*Document PCR protocol for your transgenic construct in the folder "Birth Certificates", including the information about the sequence of Primers, the size of PCR products, PCR reaction, and Programs.