

**THE NORTHWESTERN UNIVERSITY**

**TRANSGENIC AND TARGETED MUTAGENESIS LABORATORY**

###### Transgenic Project Request Form: Transient Transgenesis

Date Submitted:

Principal Investigator:

Department:

Phone:

Email:

Lab contact:

Lab contact email:

Lab Location:

Phone:

Project Initiation requirements

* Animal protocol number: an approved animal protocol describing the use of animals generated by the TTML is required before microinjections can be scheduled
* Our staff will perform the final purification of your injection DNA. Please provide a restriction digest containing ≥50µg of your **transgenic fragment (see below for details).**
* Evidence that your genotyping assay is sensitive enough to detect a single copy transgene. Once we have purified the transgenic band, a sample will be provided so that you can:
  + prepare copy standards to demonstrate the sensitivity of your genotyping PCR (<http://www.med.umich.edu/tamc/spike.html>)
  + verify that the correct band was isolated
* For more information, visit our website: [TTML transgenic projects](http://cgm.northwestern.edu/cores/ttml/transgenic-projects/index.html)

Animal protocol information

NUACUC#:

CCM animal housing location:

External investigators: please attach or forward your current IACUC approval letter from your institution.

**Mouse strai****n requested for microinjection**

B6SJL  FVB  C57BL/6

special strain (please list strain and justification):

**Has the receipt of pregnant females from our facility been added to your approved animal protocol?**

Yes  No

**Please specify the embryonic day required:**

**Project Information**

**Scientific Background**

Please provide a BRIEF description of the scientific rational for this project. Include a description of the relevant and/or unique features of the transgenic construct, and the expected pattern of expression and phenotype. If more than one construct is involved in this project, indicate how they differ and why each is significant to the project).

Transgene Structure

Transgene name:

Insert into this document a linear map of the transgene and label features including all regulatory elements (promoter, enhancer, stop and start sites for transcription, stop and start sites for translation), genomic/ reporter genes, intron/exon boundaries; relevant restriction sites; and probe/primer location used for transgenesis screening. Indicate the size of each element.

##### Regulatory elements within transgene:

1. promoter/enhancer and source: 
   1. intronic sequence and source:
   2. polyA source:

Is this regulatory element known to have tissue specific expression in other transgenic mice? If so, which tissues:

Have you demonstrated transgene expression in another system such as transfected cells?

##### Microinjection DNA

One of the most critical factors affecting integration efficiency is microinjection DNA purity. The TTML is now performing the final DNA purification step.

Enzymes used to isolate transgenic fragment from vector:

Amount of vector sequences remaining in the transgenic fragment:

Size of transgenic fragment:

Concentration and volume of restricted DNA:

* Our staff will perform the final purification of your injection DNA. Please provide the following:
  + Perform a restriction digest on your transgenic construct to generate ≥50µg of the **transgenic fragment.** Inactivate the enzyme when digest is complete.
  + Run 100ng of the restricted transgenic DNA on a gel with the appropriate molecular weight markers to insure the digestion is complete and the bands are the expected size.
  + Take a picture of the gel and clearly label the transgenic band to be isolated for injection, as well as the appropriate size markers. Insert picture of the gel below.
  + Bring the picture and the rest of the digest (in ~100-150µl volume) to the Transgenic Lab (Lurie B217).
* Once we have purified the transgenic band, a sample will be provided so that you can:
  + prepare copy standards to demonstrate the sensitivity of your genotyping PCR (<http://www.med.umich.edu/tamc/spike.html>)
* Insert the picture of the restricted DNA here.

**Genotyping for Transgene Integration**

Provide the following information about your screening assay and evidence that the assay is sensitive enough to detect a single copy transgene. To produce a single copy template, spike non-transgenic mouse genomic DNA with plasmid DNA. The following is a useful link for calculating copy number standards (<http://www.med.umich.edu/tamc/spike.html>)

PCR analysis

Primer location (also indicate position on construct map):

Expected transgenic fragment size:

* 1. Provide evidence that the transgene can be detected in genomic DNA.

Upload or insert photograph of PCR gel demonstrating sensitivity and specificity of screening assay.

# Expected Phenotype of Founder Mice

Describe any expect embryonic lethality, neonatal difficulty or death, or birth defects and provided detailed information regarding special precautions when handling pups:

##### Acknowledgements

Please acknowledge the Transgenic and Targeted Mutagenesis Facility in the acknowledgement section if you publish results using the mice that we help you generate. It is essential for our continued funding and success.

Suggested Text: “The genetically engineered mice were generated with the assistance of Northwestern University Transgenic and Targeted Mutagenesis Laboratory.”

If you are a cancer center member, please also add:

“The Northwestern University Transgenic and Targeted Mutagenesis Laboratory is partially supported by NIH grant CA60553 to the Robert H. Lurie Comprehensive Cancer Center at Northwestern University.”

**Publications**

Please provide reprints or list of publications resulting from work completed by the facility.