

**THE NORTHWESTERN UNIVERSITY**

**TRANSGENIC AND TARGETED MUTAGENSIS LABORATORY**

###### BAC Transgenic Project Request Form

Date Submitted:

Principal Investigator:

Deparment:

Phone:

Email:

Lab Contact:

Lab Contact email:

Lab Location:

Phone:

**Project initiation requirements**

* Approved animal protocol describing the use of animals generated by the TTML before microinjections can be scheduled
* Purified microinjection BAC DNA: ~20µg of linear high quality microinjection DNA ([Protocol](http://www.cgm.northwestern.edu/docs/ttml/Prep_BAC_DNA_Microinjection.pdf)), analyzed by pulse-field gel electrophoresis for purity and concentration, diluted in injection buffer at a concentration of ~50µg/ml
* Recieved microinjection DNA will be analyzed by pulse-field gel electrophoresis for purity and concentration and test injected. A new preparation will be requested if DNA is: 1) too dilute , 2) too difficult to inject (ie, clogs needles), or 3) toxic (fewer than an acceptable number of injected embryos continue to develop in culture).

Animal protocol information

NUACUC# and approval dates:

CCM animal housing location (if transferring mice within NU):

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

**Mouse strain requested for microinjection**

B6SJL  FVB  C57BL/6

special strain (please list strain and justification):

**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

Construct name:

Attach (or insert diagram below) a linear map of the transgene and label features including relevant restriction sites and probe/primer location used for transgenesis screening.

##### Transgene preparation

The two most critical aspects of successfully creating transgenic founders from BAC transgenes are 1) purity of DNA and 2) DNA concentration. The recommended protocol for preparing high quality BAC DNA for microinjection [(Protocol)](http://www.cgm.northwestern.edu/docs/ttml/Prep_BAC_DNA_Microinjection.pdf) is that of Nathaniel Heintz. We have also been successful at generating BAC transgenic founders from DNA prepared using [Clontech's NeocleoBond BAC Maxi Kit.](http://www.clontech.com/AIT/Ecommerce/Clontech/productCatalog.aspx)

Circular or linear BAC DNA can be submitted for microinjection. Pulsed field analysis is critical to determine the quality of the DNA as this method detects sheared BAC DNA or accidentally restriction enzyme digested BAC preps that will never result in the generation of founders. We will assess the submitted BAC DNA by PFGE prior to injection. A sharp, single band of the correct size should be evident.

Enzymes used to linearize microinjection BAC:

Size of purified BAC injection fragment:

Form of injection fragment  circular

linear

Concentration (should be ~50g/ml) and volume of purified DNA:

Genotyping

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 10mg of mouse DNA with 2pg of plasmid DNA for every Kb of the control construct.

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.

Attach photograph of PCR gel or autoradiograph demonstrating sensitivity and specifcity of screening assay.

# Phenotype

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.

Billing Information

Date:

Project Name:

Principal Investigator:

Department/Division:

Lab Contact:

Lab Contact Phone:

Lab Contact Email:

PI signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Grant Title:

Grant Number:

Funding Agency:

CUSF or PO#:

Full Cancer Center Member:  yes  no

Accounting Contact Name:

Accounting Contact Phone:

Accounting Contact Email: