

TRANSGENIC AND TARGETED MUTAGENESIS LABORATORY (TTML)

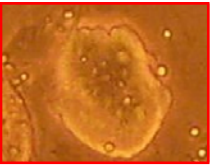
The Northwestern University Transgenic and Targeted Mutagenesis Laboratory (TTML) is a shared resource designed to produce genetically engineered mice for research projects of investigators on the Evanston campus, in the Feinberg School of Medicine, the Robert H Lurie Comprehensive Cancer Center, Evanston Northwestern Hospital (ENH), and Children's Memorial Research Center (CMRC). The transgenic laboratory was first organized at Northwestern University in 1989 as part of the Markey Program in Developmental Biology, to provide a resource for generating transgenic mice by pronuclear microinjection. Today, the facility has evolved into a highly utilized laboratory that provides a broad range of services to NU investigators:

Generation of transgenic mice [\(see video\)](#)

Plasmid-based or BAC derived transgenic constructs containing all the genetic elements necessary for their expression and translation into functional proteins are designed by the investigator and provide for microinjection. Purified transgenic DNA, microinjected into the pronucleus of a single-cell embryo, integrates randomly into the genome of some injected embryos. Microinjected embryos are then surgically into surrogate mothers and pups are born 19 days later. Typically, many copies of the transgene incorporate into the genome during the integration event, ultimately resulting in high levels of transgene product. These transgenic gain-of-function models can serve as a good experimental method for studying the effects of altering gene expression in a particular tissue and time during development.

Targeted mutagenesis or gene targeting

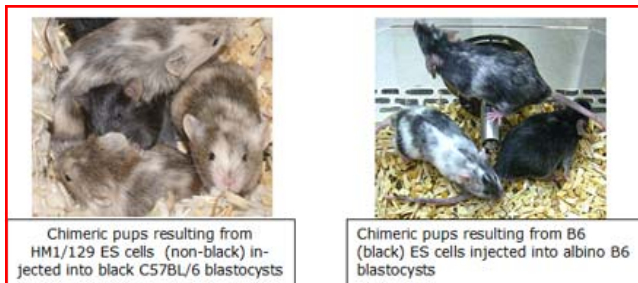
Genetic diseases can be caused by a minor change (i.e., a single-point mutation) in a gene or a larger mutation that results in either a decrease in or the complete loss of that gene's function. Gene-targeting methods can be used to introduce such site-specific mutations in particular mouse genes to more faithfully mimic human disease. Key to this process is the ability to manipulate genes *in vitro* using pluripotent stem cells derived from early preimplantation embryos.



The laboratory maintains pluripotential HM1 (129/Ola) embryonic stem (ES) cells that have been tested for their ability to give rise to most tissues of a mouse including germ cells. Investigator-designed targeting vector DNA electroporated into ES cells specifically replaces endogenous gene sequences of identity recombines at low frequency via homologous recombination. Electroporated clones that survive *in vitro* selection are analyzed by the investigator for the targeting event. Targeted clones that have incorporated the DNA by homologous recombination are expanded and microinjected into blastocysts to create chimeric mice.

ES cell microinjection into blastocysts [\(see video\)](#)

Mice carrying a site-specific mutation are produced by injecting targeted ES cells into the cavity of an expanded 3.5d pre-implantation stage blastocyst. The microinjected targeted ES cells incorporate into the cells within the blastocyst that give rise to all the tissues of the embryo, the intercell mass (ICM). Injected blastocysts are then surgically transplanted into female recipient mice. Among the resulting litters are colorful chimeric pups with tissues, including germ tissue, composed of cells derived from both the



Chimeric pups resulting from HM1/129 ES cells (non-black) injected into black C57BL/6 blastocysts

Chimeric pups resulting from B6 (black) ES cells injected into albino B6 blastocysts

targeted ES cells and the host blastocyst. The degree to which the targeted ES cells populate the ICM during injection is evident by the coat color of the chimera: all skin and hair derived from ICM cells of the host blastocyst will be a different color (ie, black) than that arising from the ES cells (ie, white/agouti/tan).

Since relocating to the Chicago campus, the laboratory has generated knockout lines for 22 projects; 18 using HM1 (129/Ola) ES cells and 4 using C57BL/6 derived ES cell lines. Chimeric mice have also been successfully generated for investigators supplying from investigator supplied ES cells derived from R1, E14TG2a, GS-1 and AB2.2.

Rederivation

The process of rederivation eliminates pathogens such as pinworm, parvovirus, and mouse hepatitis virus from infected mouse lines.



Pre-implantation embryos, which are relatively resistant to such pathogenic microorganisms due to the physical barrier of the zona pellucida surrounding the embryo, are collected for from infected mice and washed through several droplets of embryo culture media then surgically transferred into specific pathogen-free pseudopregnant females. Resulting mice can then be transferred to the new barrier-level vivarium in the Lurie Research Center.

Cryopreservation and cryorecovery

Cryopreservation is the process of freezing viable embryos for stable, long-term storage in liquid nitrogen.

It is a safe method for preserving viable embryos that can be easily recovered and transferred to pseudo-pregnant females to revive the line. It is a mainstream technique used by all major mouse repositories (e.g., Jackson labs, MMRRRC, KOMP, etc.) and most major research institutions. It provides an economically feasible and sustainable method for managing the huge repertoire of genetically altered mice that are currently available to investigators. Investigators supply males for cryopreservation. Pre-implantation embryos are then generated, collected, frozen, and stored in liquid nitrogen freezer dewars. "Speed-cryogenic"

methods can be used for mouse strains amenable to *in vitro* fertilization (IVF) such as C57BL/6. In this method, hundreds of eggs are fertilized via IVF using only a couple of males.

Cryopreserved embryos is a safe, affordable way to transport mouse lines worldwide, eliminating the shipment of live animals. It is also an acceptable method for rederiving (to rederive) infected mouse lines. TTML has capability of both shipping and receiving cryopreserved embryos. Frozen embryos received from outside the institution or stored in the TTML can be thawed and transferred into recipient females (cryorecovered) at any time.

The laboratory is establishing a stock of cryopreserved mouse lines that will be available to all NU investigators. These lines will be cryopreserved, distributed and maintained by the TTML. Those investigators interested in donating lines should contact the facility at 312-503-0088 or TTML@northwestern.edu.

The TTML provides the necessary infrastructure to allow most investigators to access transgenic technology that normally requires expensive microinjection/tissue culture equipment. TTML staff provide consultation on all aspects of transgenic related technologies ranging from animal protocol approval to breeding, screening and analysis of newly created transgenic or chimeric mice. TTML staff is also available to provide guidance regarding targeting vector design, appropriate screening strategies, and DNA isolation methods for gene targeting projects.