CONTACT US

Location:

Colket Translational Research Building
Level C, 700 Hallway
Animal Room, C-705
Procedure Room, C-703

Staff:

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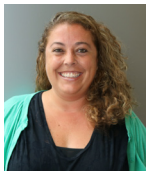

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Children's Hospital of Philadelphia
RESEARCH INSTITUTE

Transgenic Core

A state-of the-art transgenic mouse core facility serving members of the hospital's research community.

Performing procedures such as; DNA, CRISPR/Cas9 and ES cell microinjections/electroporations, embryo and sperm cryopreservation, IVF, and mouse line rederivation.

Colket Translational Research Building
(CTRB) C Level, 700 Hallway
Animal Room - 705
Procedure Room - 703

WHAT SERVICES DO WE OFFER?

The facility is located on C-level within the barrier 700 hallway of the Colket Translational Research Building.

The facility is equipped to perform a number of procedures such as;

- DNA microinjection
- CRISPR/Cas9 microinjection/electroporation
- ES cell microinjections
- Embryo and sperm cryopreservation and storage
- In Vitro Fertilization (IVF)
- Mouse line rederivation/rescue.

DNA MICROINJECTION

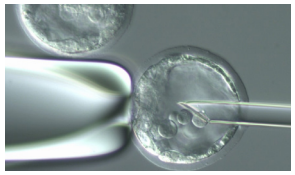
Insertion of prepared constructs takes place with the aid of a micromanipulator and microinjector. The DNA solution is injected directly in the pronucleus of wild-type 1-cell embryos (0.5dpc) zygotes. These are then surgically transferred into surrogate mother mice, where they will develop to term.

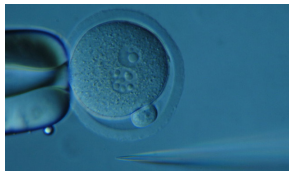
The core will try to accommodate any background strain requested, but some may be more successful than others, so the number of founders generated will likely vary.

CRISPR/CAS9

CRISPR technology can greatly reduce the traditional cost and time needed to generate knockout/in models for scientific research. The CRISPR system is currently being use to generate the following mutations: KO, CKO, KI, simultaneous targeting, epitope tags and new mutations in existing mutant strains.

Insertions of the RNA takes place with the aid of the electroporator or the micromanipulator and microinjector, and depending on the concentration can either be injected into the cytoplasm or the pronucleus of 1-cell (0.5 dpc) zygotes.





ES CELL MICROINJECTION

Gene targeting is carried out in mouse embryonic stem (ES) cells. ES cells are derived from very early, usually male, mouse embryos and have the capacity to contribute to the complete development of the animal. Targeting works by isolating the gene sequence and then replacing it with a version of the same gene, in which a region has been deleted or a mutation has been inserted.

The core microinjects approximately 60-80 blastocysts (3.5dpc) for each ES cell line/clone. We typically inject 8-15 ES cells/blastocyst.

CRYOPRESERVATION

Cryopreservation provides a useful tool for archiving mouse lines. The purposes of embryo and sperm cryopreservation are to:

- Protect against the loss of valuable strains through breeding failure, disease, human error, etc.
- Eliminate the cost of maintaining inactive mouse lines.
- Make available space for other mouse lines.
- Importing/exporting strains to/from collaborators.
- Facilitate future establishment of mice into new barrier facilities.

Frozen specimens can be frozen in liquid nitrogen indefinitely, as long as they are maintained appropriately.

REDERIVATION

Mouse line rederivation is the method used to eliminate pathogenic or potentially pathogenic agents. This procedure involves transferring pre-implantation stage embryos into recipient surrogate mothers (with known health status).

Investigators may occasionally require the use of this service if their strain is no longer producing offspring. There are a number of methods we can use to help you recover/rederive your strain, so please feel free to contact us.