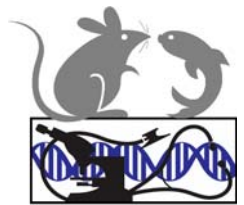


# 2006 Johns Hopkins Phenotyping Symposium

## Reeves Tg Core: ES cell to Mouse



### Transgenic Core Laboratory

The core is a comprehensive facility which provides genetic engineering services in mice to the entire Johns Hopkins University community.

The core is subsidized in part by the Institute for Basic Biomedical Sciences; support for expansion of ES cell services comes through a Core support grant from NINDS (Alex Kolodkin)

Roger Reeves: Faculty Director  
 Charles Hawkins : Facility Director  
 Ann Lawler : Microinjectionist  
 Johnisha Witherspoon : Microinjectionist  
 Shontia Karim: Animal Technician  
 Holly Wellington: ES cell specialist

<http://www.med.jhu.edu/CORE/Home.htm>

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### Transgenic Core Services

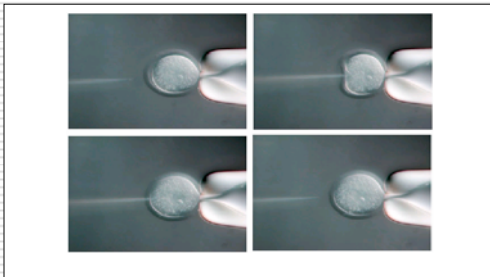
1. Pronuclear injection to create transgenic mice
2. Blastocyst injection of ES cells to produce chimeric mice
3. Cryopreservation of Embryos
4. Cryopreservation of sperm
5. Reconstitution from frozen sperm or embryos
6. Rederivation of pathogen-contaminated strains
7. Ploidy analysis
8. Special breeding/husbandry advice

#### NEW! ES cell services

1. Two ES lines available for purchase
2. Gene-targeting of your construct into ES cells
3. Derivation of ES cells from your mice

<http://www.med.jhu.edu/CORE/Home.htm>

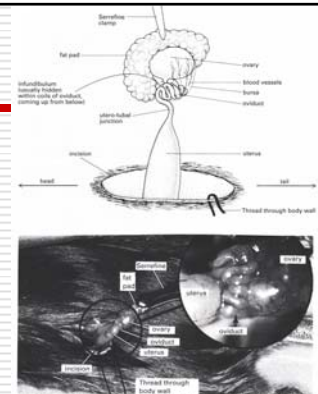
### Pronuclear injection: Production of Transgenic Mice



<http://www.med.ic.ac.uk/db/dbbm/tgunit.htm>

### Production of Transgenic Mice

A 28 ga needle is used to introduce injected zygotes into the uterus of a pseudo-pregnant recipient. Pups are born ca. 19 days later.



Preimplantation mouse embryos. Hogan et al., Manipulating the mouse embryo, 1994.

### Transgenic Mice can be used to:

1. Model dominant genetic conditions (by building in mutations)
2. Create genetically marked populations of cells
3. Survey genome regions (cryptic functions)
4. Promoter bashing

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### Production of Transgenic Mice

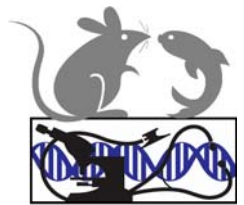
#### Timeline:

- Investigator provides a linearized DNA construct to the core at which time the construct is placed in the injection queue
- Three weeks after injection, pups are born
- Three weeks after birth, the investigator screens for founders; mice are transferred to his/her colony

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## Reeves Tg Core: ES cell to Mouse



### Production of Transgenic Mice

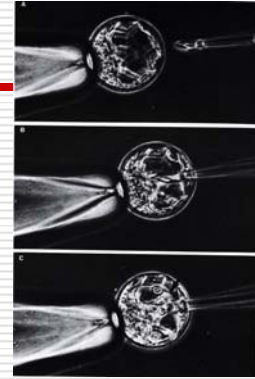


- The core performs pronuclear injection to generate transgenic mice
- For each DNA construct, a minimum of 2 founders will be provided
- The standard fee is \$3500 for all mouse and injection costs
- BAC transgenics, special strains, the fee is \$4500 and extra mouse charges may apply depending on the strains selected

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### Blastocyst Injection: Production of Gene- targeted Mice

Embryonic stem cells can be manipulated in culture, then injected into mouse blastocysts to form part of the inner cell mass (ICM). The ICM will form the embryo proper while the surrounding trophectoderm cells contribute to extra-embryonic structures (placenta).



<http://www.med.jhu.edu/CORE/Home.htm>

### Production of Gene-targeted Mice



A chimera has four parents, but individual cells have genetic information from only one of the two pairs.

<http://www.med.jhu.edu/CORE/Home.htm>

### Production of Gene-targeted Mice: Engineering in ES cells

1. Model recessive genetic conditions ("knock in")
2. Create genetically marked populations of cells
3. Find expressed genes (Promoter/enhancer trap)
4. Express genes conditionally (specific times and places)
5. Mutation library

<http://www.med.jhu.edu/CORE/Home.htm>

### Production of Gene-targeted Mice



- The core injects *euploid* ES cells into mouse blastocysts to produce chimeric mice
- 4 days of injections for up to 3 clones are scheduled
- The standard fee is \$3500 per construct for mouse and injection costs

<http://www.med.jhu.edu/CORE/Home.htm>

### Production of Gene-targeted Mice

#### Timeline:

- The investigator identifies targeted ES cell clones and determines ploidy
- Schedule an injection date for the clones with the core
- Three weeks later pups are born
- 7-10 days later, chimeras are recognizable by coat color
- Mom and pups transferred to BRB

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## Reeves Tg Core: ES cell to Mouse



### Gene-targeting in ES cells

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- The investigator provides a construct to the Core
- ES cells are electroporated and clones expanded under selection
- Colonies are picked and expanded for DNA extraction
- DNAs from drug-resistant clones are provided to the investigator (cell lines remain frozen in the Core)
- The investigator screens for correctly-targeted clones
- The Core expands positive clones, screens for ploidy, and puts euploid cells in the queue for chimera production

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### Other services

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- Cryopreservation of sperm and embryos
  - a) get unused mouse lines off the shelf
  - b) sometimes a good way to ship "mice"
- Rederivation of contaminated mouse strains
- ES cell derivation (ES cell lines available at cost) and ploidy
- Ovary transplant
- ES cell expansion, e.g., of BayGenomics ES cells
- In vitro fertilization

- PROVIDE **ADVICE**

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<http://www.med.jhu.edu/CORE/Home.htm>

### Contact the Transgenic Core

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<http://www.med.jhu.edu/CORE/Home.htm>

Phone: (410) 614-3858

Fax: (410) 614-9745

[chawkins@jhmi.edu](mailto:chawkins@jhmi.edu)

[rreeves@jhmi.edu](mailto:rreeves@jhmi.edu)

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<http://www.med.jhu.edu/CORE/Home.htm>