



The University of Michigan Transgenic Animal Model Core

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Mouse Pronuclear Microinjection Training Syllabus

Purpose:

To provide all knowledge and hands-on experience necessary to perform pronuclear injection of fertilized mouse eggs and to produce transgenic mice. Trainees may bring a transgene.

Overview:

Training is divided into three phases:

1. Discussion and lab experience on fertilized egg collection and transfer to pseudopregnant female mice.
2. Discussion and lab experience in microinjection of mouse embryos and transfer to pseudopregnant female mice.
3. Follow-up on pregnancies and genotyping

Preparation:

1. Trainees will read and be familiar with the following sections in *Manipulating the Mouse Embryo: A Laboratory Manual*. 3rd ed. 2003. Nagy, Gertsenstein, Vintersten and Behringer. Cold Spring Harbor Laboratory Press. pp. 141-197; pp. 251 – 257; pp. 289 – 320; pp. 332-358.
2. Practice in the fabrication of glass instruments used in transgenic mouse production.
3. Practice in the use of mouth pipettor and transfer pipets used to move mouse eggs.

Schedule:

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|-------|---|
| Day 1 | 8:00- 5:00 fabrication of glass micro-instruments. |
| Day 1 | 8:00 - 5:00 collect eggs and transfer eggs to recipients |
| Day 2 | 8:00 - 5:00 workstation orientation, collect and microinject eggs |
| Day 3 | 8:00 - 5:00 collect eggs, microinject, and transfer injected eggs to recipients |
| Day 4 | 8:00 - 5:00 collect eggs, microinject |
| Day 5 | 8:00 - 5:00 collect eggs, microinject, and transfer injected eggs to recipients |

In order to derive the maximum benefit from this training, trainees should plan to spend at least a full day (8 hours) in the Transgenic Core on scheduled training days.

Follow-up:

Egg transfer surgeries from the first two sessions will be evaluated in terms of the number of pregnancies and implantations that occur. Trainees will prepare tail DNA from any mice born from their microinjected eggs and determine if they are transgenic. Transgenic founders will be transferred to the trainee, at his/her request. This information will provide the means to evaluate the effectiveness of the training.

Discussion Papers

Auerbach AB, Norinsky R, Ho W, Losos K, Guo Q, Chatterjee S, Joyner AL. 2003. Strain-dependent differences in the efficiency of transgenic mouse production. *Transgenic Res.*12:59-69.

Brinster RL, Chen HY, Trumbauer ME, Yagle MK, Palmiter RD. 1985. Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc Natl Acad Sci U S A.* 82:4438-4442.

Taketo M, Schroeder AC, Mobraaten LE, Gunning KB, Hanten G, Fox RR, Roderick TH, Stewart CL, Lilly F, Hansen CT, Overbeek PA. 1991. FVB/N: an inbred mouse strain preferable for transgenic analyses. *Proc Natl Acad Sci U S A.* 88:2065-2069.

Van Keuren ML, Gavrulina GB, Filipiak WE, Zeidler MG, Saunders TL. 2009. Generating transgenic mice from bacterial artificial chromosomes: transgenesis efficiency, integration and expression outcomes. *Transgenic Res.* 18:769-785.

Additional Resources

Advanced Protocols for Animal Transgenesis: An ISTT Manual. 2011. Pease, S and Saunders, TL (eds). Springer, New York.