Animal Source

PSU Animal Resource Program

Volume 5, Issue 1
Winter 2007

Mouse Breeding Basics

Mice can be easy and prolific breeders with the ability to produce many offspring over their lifetimes. However, a basic knowledge of mouse biology is necessary for optimum reproductive success with laboratory mice.

Female mice reach sexual maturity between 6-10 weeks of age. Outbred females tend to mature earlier than females of inbred strains. Female mice are typically retired from breeding by one year of age. Male mice reach sexual maturity at around 5-6 weeks of age and can be fertile throughout their lives.

Sexually mature female mice have an estrus cycle that lasts 4-5 days and will be receptive to mating with a male at the estrus stage of the cycle. Because females can be territorial and resist intruders, it's best to place the female in the male's cage for breeding. Estrus and mating usually occur at night. A yellowish to white colored plug may be visible in the female vaginal opening for several hours after mating. The plug is an indication that mating has occurred and may be used to date the start of the pregnancy. Because they fall out quickly, the absence of a plug is not significant. Gestation lasts between 19-21 days and varies between individuals and strains.

The most common mating arrangements for mice are the permanent breeding pair and the breeding trio. In the breeding pair, one female is placed with one male. The pair remains together throughout all subsequent pregnancies. Breeding pairs can produce a litter approximately every 21 days due to a post-partum estrus period that occurs 14-24 hours after a litter is born. However, concurrent lactation may delay embryo implantation in certain strains of mice and prolong the subsequent gestation period for up to 12-13 days.

In the breeding trio arrangement, two females are placed with one male. The pregnant females may remain in the cage with the male permanently or they may be removed from the male's cage before giving birth and placed in cages of their own. If removed, the male is then provided with two new females. This cycle is repeated as many times as necessary. The trio arrangement results in greater numbers of offspring per cage (or male). A greater number of offspring per female is obtained using the permanent breeding pair arrangement.

Visit the ARP website at www.research.psu.edu/arp for answers to your animal use questions

Inside this issue:

Surgical Records for Research Animals

Surgical records are an important part of research animal care. Good records document the procedures performed and perioperative care provided and communicate that information to other professionals. Surgical records should provide sufficient detail so that the surgical and peri-operative process can be reconstructed, if necessary. Records must be readily available for review by the attending veterinarian and others involved in internal (e.g., IACUC) or external (e.g., AAALAC) oversight.

Individual records must be maintained for nonrodent species but groups of rodents treated similarly may be combined on one record. Surgical records must include:

- The name of the surgeon and the date surgery was performed.
- Animal identification i.e., number or group name
- A description of the surgical procedure(s) conducted. Note: A complete description of the procedure is not necessary for every record. The name of the procedure is sufficient when the identical procedure is performed on multiple animals provided that an SOP (standard operating procedure) describing the procedure is readily available.
- Name(s), dosage(s) and route(s) of anesthetic and analgesic agents administered.
- Names and dosages of experimental agents administered.
- Post-operative monitoring that indicates daily observation and health status.
- Medical treatments administered during or after surgery.

All entries in the record should be written in permanent ink, dated and indicate the originator of the entry via initials or signature. Entries must be legible to someone other than the writer. Corrections to previous entries should be dated and initialed with the old entry crossed out using a single line.

Congenic Strain Development in Mice: Backcrossing and Speed Congenics

Genetically engineered mice, such as transgenic and knockout mice, are commonly created using either FVB/N or 129 mice as the background strain. Although there are legitimate reasons why these two strains are used, they are not always the best background strain choice. To help resolve this problem, researchers can transfer the transgene or knockout allele to a different, more appropriate genetic background through the development of a congenic strain.

Congenic strains are created through the process of backcrossing. In traditional backcrossing, transgenic or knockout offspring are sequentially bred to mice of a recipiant background (i.e., inbred strain) for 10 generations. The result is a mouse strain genetically identical to the recipient strain except for the transgene or knockout allele (and a small amount of residual genetic material surrounding that locus). The congenic strain will have less than 1% of the original background strain remaining in the genome. Creation of a congenic strain through traditional backcrossing typically takes 2-3 years.

A faster method of developing a congenic strain involves using marker assisted selection of breeders. In this process, known as speed congenics, transgenic or knockout offspring are sequentially bred to mice of a recipient background as in traditional backcrossing. However, offspring carrying the transgene or knockout allele are subjected to further screening for genetic background composition at various microsatellite DNA marker loci. Microsatellites are repetitive short sequences of DNA with an identifiable physical location on a chromosome and whose inheritance can be followed. Strain variations in microsatellites have been identified and can be used as unique markers to identify the genetic background source of chromosomal regions.

By selecting for breeding offspring who have a higher percentage of genetic material from the recipient background strain a congenic strain can be created within 4-5 backcross generations. It takes approximately 18 months to develop a congenic strain using the marker assisted selection protocol.

However, due to the increased need for genetic screening, speed congenics requires a significantly greater number of mice and genotyping procedures. Investigators must determine if the decreased development time outweighs the increased production costs. Several commercial genetics companies offer services to facilitate congenic strain development. These companies can provide all colony management and genotyping services for the entire speed congenics process or just provide genotyping services for customers housing animals in their own facilities.

More information on backcrossing and speed congenics may be found at the following sources:

Charles River Laboratories. Marker-Assisted Accelerated Backcrossing (MAX-BAX^{SM}). Online at http://www.criver.com/research_models_and_services/genetic_testing_services/acceleratedbackcrossingspeedcongenics.html.

Harlan Speed Congenics Services. Online at http://www.harlan.com/index6.asp.

The Jackson Laboratory. Speed Congenic Development Service. Online at http://jaxmice.jax.org/services/speedcongenic.html.

Wong GT. Speed congenics: Applications for transgenic and knock-out mouse strains. *Neuropeptides* (2002) **36**(2-3), 230-236.

The next Mouse Biomethodology Seminar will be held on February 2, 2007 from 1-4 pm in the Centralized Biological Laboratory. Please call 865-1495 to register to attend.

Animal Resource Program

101 Centralized Biological Laboratory
Pennsylvania State University
University Park, PA 16802

(814) 865-1495
Fax: (814) 865-3685

A sheep sold at the PSU April 2006 sale to Superior Farms of Oklahoma City, OK was awarded Reserve National Champion Dorset Female at the 2006 North American International Livestock Expo this fall.

The Animal Resource Program (ARP) is committed to providing PSU research personnel with high quality animal care services and facilities, to facilitate and improve animal research, and to ensure the health, well-being and humane treatment of all animals at PSU. ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal, agricultural and wildlife technology and medicine. ARP veterinarians have specialized training and are available to assist with animal model development, experimental design, budget projections and grant preparation. Participation in collaborative research projects is welcomed.

Nature Survey of Scientists' Attitudes to Animal Research

In October 2006 the journal *Nature* conducted on online survey of scientists' attitudes to the use of animals in research. Survey responders were registered readers of the journal who anonymously provided answers to the questions. One thousand six hundred eighty two people responded.

- Greater than 80% of the responders resided in the United Kingdom, Europe or North America (including Canada).
- Approximately 50% used animals in their experimental work.
- Of those who conducted research with animals, more than 80% used mice and rats.
- Of those who conducted research with animals, 80% had no "ethical misgivings" about the role of animals in their research.
- 61% of those using animals in research had not discussed their work with the general public (either directly, i.e., public lectures, or via the media).
- Of all responders, 71% thought more discussion about the use of animals in research was needed.
- 74% thought animal research was "essential" for progressing biomedical science.
- 62% saw the "elimination of animal experiments" as a "desirable but unachievable goal".
- 70% thought the "legislation governing the use of animals in research" in their country was "about right".
- 71% believed that "the animal rights movement makes it difficult for a researcher to voice a nuanced opinion on animal research (for example, one that supports most research but has reservations about some of it)".

Nature. Vol 444, December 14, 2006. http://www.nature.com/news/specials/animalresearch/index.html