

# Research Resources

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Welcome to the first issue of *Research Resources*, your one stop for news, updates, and happenings at the animal care facilities. In this and following issues we hope to keep you informed of changes in policies and personnel as well as new technologies and opportunities. This will be sent out to all Active Project Directors, but I encourage you to share this with all your staff, and, if any want to be included in the mailing, have them send a request to [stacy.wells@louisville.edu](mailto:stacy.wells@louisville.edu). So, without further ado, the first edition:

**INTERNATIONAL LABORATORY  
ANIMAL TECHNICIAN  
APPRECIATION WEEK  
January 29<sup>th</sup> to February 4<sup>th</sup>**

During the 1999 American Association for Laboratory Animal Science (AALAS) National Meeting in Indianapolis, a National Laboratory Animal Technician Week was created in honor of AALAS' 50th year of existence. This annual celebration recognizes laboratory animal technicians for their essential contributions as members of the research team. Because of its success, it was expanded in 2002 and renamed the International Laboratory Animal Technician Week, allowing technicians from around the world to participate in the event. AALAS and the International Council for Laboratory Animal Science (ICLAS) work together to acknowledge technicians for their roles in the biomedical/biological research field.

The Research Resources Facilities are open seven days a week, 365 days a year. The Animal Technicians are an invaluable part of the research effort here at the University. This dedicated group of animal health professionals provides daily husbandry, health monitoring, and research support. The Animal Technicians are very often the first to recognize any variables that may affect a research project, such as signs of illness or environmental factors. The Animal Technician staff is responsible for the day-to-day husbandry and health monitoring of the animals. Researchers can be assured that their animals are provided with clean cages/bedding, water and food, and that the animal's environment is monitored and maintained at an appropriate level. They observe every animal every day for signs of unusual behavior or illness. This year's theme is "I

do the caring for you." Emphasizing the role that the animal technician has in easing your minds that the animals that are so dear to your research are well provided for.

We hope this year that you and your research team will join the Research Resources Administration in showing our appreciation for the work that these technicians do to make the research go more smoothly. The efforts of these hard-working professionals are an integral component of the successful research team, and I encourage everyone to show their appreciation for their valuable contributions – not only during International Laboratory Animal Technician Week, but everyday. We couldn't do it without them!

**WHAT'S AALAS, YOU SAY?**

Well, since I brought it up, I might as well tell those of you who have not heard of or know much about it. The American Association of Laboratory Animal Science is the premier forum for the exchange of information and expertise in the care and use of laboratory animals. Since 1950, they have been dedicated to the humane care and treatment of laboratory animals and high-quality research that leads to scientific gains benefiting people and animals. The core value statement of AALAS is, "The American Association for Laboratory Animal Science believes that the use of laboratory animals in scientific and medical research is essential to the improvement and protection of the quality of all life. The humane and responsible care of laboratory animals is vital to quality research and, as such, an essential aspect of AALAS endeavors. AALAS is dedicated to building and disseminating a knowledge base in laboratory animal science for the education and training of those who work in this field."

AALAS publishes journals and has meetings to distribute this knowledge. There are opportunities for research staff to participate both locally and nationally. To learn more about National AALAS visit [www.aalas.org](http://www.aalas.org) and to learn more about the Kentucky Branch of AALAS please ask any of the facility supervisors or visit [www.ky.d5aalas.org](http://www.ky.d5aalas.org).

On a local note, the Kentucky Branch of AALAS recently won the Branch Challenge at the National Meeting in St. Louis, MO. The Branch Challenge Award goes to the Branch with the highest percentage increase of National Membership in AALAS. This was achieved by the number of technicians at U of L that continue to give the most dedicative caring by keeping up with new technologies and innovations through the many opportunities offered by AALAS.

## TAIL CLIPPING

### Medical Information:

The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nervous tissues. In a young mouse (<21 days) the tissue near the tip of the tail is soft and the bones have not completely mineralized. Therefore, removing of the tail tip of a young mouse probably amounts to only momentary pain for the animal. As the animal ages, tissue maturation includes mineralization of the bone and increased vascularity. Tail tip sampling performed on an older animal (>21 days) is likely to involve more than momentary pain and distress as well as the potential for significant hemorrhage.

It is generally believed that tail tip removal should be performed at as young an age as is feasible. In most, if not all, cases the procedure can be performed prior to weaning and there is nothing to be gained by genotyping at an older age. The following guidelines are from NIH (reference: <http://oacu.od.nih.gov/ARAC/>). The University of Louisville IACUC will soon be officially ratifying and enforcing its own guideline for the procedure.

### ***NIH Guidelines for the Genotyping of Rodents***

The proper identification of transgenic animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. Most often the genotype is determined by analysis of DNA extracted from tissues of young mice. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of

DNA. DNA for PCR analysis can be obtained from ear punches, hair samples, or oral swabs (see references 1-6). Depending on the requirements of the study, investigators are urged to consider these alternatives. Larger amounts of DNA are required for Southern Blot determination of the genotype. The ARAC (Animal Research Advisory Committee) has determined that obtaining tissue from a mouse for DNA analysis via tail biopsy is a safe, effective and humane procedure that causes minimal or transient pain and distress when performed properly.

DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR.

### ***Guidelines for Tail Biopsy***

**1.** Procedures for tail biopsy for DNA analysis and/or genotyping must be described in an approved Animal Study Proposal (ASP).

**2.** Ideally, mice should be **10-21** days old. At this age, the tail tissue is soft (vertebra are not yet calcified) and the yield of DNA is highest. In addition, prompt analysis of tail tissue allows the desired mice to be identified prior to weaning which can facilitate more efficient use of cage space.

**a. For mice 10-21 days of age:** Because pain sensory development may be complete, and to further minimize any transient pain or distress, investigators are strongly encouraged to apply local anesthesia to the tail. Local anesthesia may be achieved by immersion of the tail in ice cold ethanol for 10 seconds. Alternatively, the tail can be disinfected with 70% ethanol and allowed to dry, followed by an application of ethyl chloride spray or other suitable anesthetic as recommended by the attending veterinarian.

(An ARAC subcommittee is currently evaluating pain sensitivity specifically during tail biopsy in 10-21 day old mice. Investigators should note that this guideline is likely to become more definitive when the results of these studies are complete.)

**b. For mice greater than 21 days of age:** The use of a local or general anesthetic is required prior to collection of tissue. If a general anesthetic is to be used, an appropriate agent should be recommended by the attending veterinarian.

**3.** Manually restrain the mouse between thumb and forefinger. This is a convenient time to identify the animals using the appropriate method (i.e. ear punch, ear tag, transponder etc.).

4. With sterile scalpel, razor blade, or scissors cleanly excise the distal 5 mm of tail. If the proper procedures are followed, the yield of DNA from 5 mm of tail should exceed 50 micrograms, enough for multiple analyses. The yield of DNA does not proportionally increase as tail fragments larger than 5mm are used. If small amounts of DNA are required, investigators should consider taking only 2 mm of tail.

If the analysis of the DNA is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal. Disinfect the scalpel or scissors between animals. If a scalpel is used, also disinfect the work surface on which the tail is placed between animals.

5. The investigator must monitor the animals to assure hemostasis after the animals are returned to the cage. Apply digital pressure, silver nitrate, or other means of hemostasis.

6. Repeat tail biopsies require general anesthesia and must be justified in the ASP.

#### References

1. Hofstetter JR, Zhang A, Mayeda AR, Guscar, T, Nurnberger JI and Lahiri DK. Genomic DNA from Mice: A Comparison of Recovery Methods and Tissue Sources. *Biochem Mol Med* 1997 Dec; 62(2):197-202.
2. Dennis, MB. IACUC Review of Genetic Engineering. *Lab Animal* 2000 Mar; 29(3):34-37.
3. Irwin MH, Moffatt RJ and Pinkert CA. Identification of Transgenic Mice by PCR Analysis of Saliva. *Nat Biotechnol* 1996 Sep;14(9): 1146-8.
4. Schmitteckert EM, Prokop CM and Hedrich HJ. DNA Detection in Hair of Transgenic Mice - A Simple Technique Minimizing the Distress on the Animals. *Laboratory Animals* 1999; 33/4: 385-389.
5. Couse JF, Davis VL, Tally WC and Korach KS. An Improved Method of Genomic DNA Extraction for Screening Transgenic Mice. National Institute of Environmental Health Sciences, National Institutes of Health. *BioTechniques* 1994; 17:1030-1032.
6. Malumbres M, Mangues R, Ferrer N, Lu S and Pellicer A. Isolation of High Molecular Weight DNA for Reliable Genotyping of Transgenic Mice. *BioTechniques* 1997; 22/6:1114-1119.

### New IACUC Policy

Recently, the IACUC approved a new policy outlining training requirements for research personnel. This new policy reads:

Regulatory agencies require the University, through the oversight of the Institutional Animal Care and Use Committee (IACUC), to ensure all personnel working with laboratory animals possess appropriate experience and training. The IACUC verifies this via three mechanisms: 1) reviewing a description of each protocol participant's training and experience, 2) providing didactic sessions to inform all animal users of their roles and responsibilities, and 3) coordinating "hands-on" interaction with veterinary or other specialists, as needed.

The training available to scientists, animal technicians, and other personnel involved in animal care, treatment, observation, or use is designed to ensure that these individuals understand the principles of humane animal care and are qualified for their specific role. Such information is maintained in the IACUC database. Individuals who have not met training requirements are informed of the need to do so during the *Proposal* review process and animal use privileges are contingent upon meeting the IACUC training requirements. Furthermore, the Research Resources Facilities (RRF) requires facility-specific training before access is granted into animal vivaria.

To facilitate this requirement, the IACUC has adopted the following three planes of instruction:

#### **Level I:**

Periodically, individuals must be exposed to laboratory animals or research facilities on single occasions with no intention of working independently (*e.g.*, students involved in a teaching exercise involving animal models, project sponsors wishing to review a procedure). Although these individuals must understand their responsibilities and limitations, as well as the hazards associated with research animal contact, the depth of training necessary is minimal. "Level I Training" consists of the informative brochures entitled, "The Use of Animals in Biomedical Research at the University of Louisville," and "Introduction to Occupational Hazards Associated with the Care and Use of Laboratory Animals." These handouts are provided by the RRF to visiting

personnel who must remain under the supervision of a Level II trained staff member. Review of these documents, which provide a summary of basic tenants, authorizes individuals to work with animals, during regular working hours only (Monday through Friday, 8:00 AM to 4:30 PM), only under direct supervision and does not confer unsupervised access to animal facilities. Posted notices and sign-in sheets to document receipt of this information will be maintained at RRF entrances.

### **Level II:**

The basic training program for personnel wishing to work independently with laboratory animals is known as “Level II Training,” which is composed of session presented monthly (special presentations may also be arranged). The seminar outline follows the recommendations of the National Research Council (NRC) publication, *Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs*. The discussion includes an overview of pertinent animal welfare recommendations, regulations, and regulatory bodies, such as:

- Russell, WMS, and RL Burch, *The Principles of Humane Experimental Technique* (1959) and an introduction to the concepts of “The Three R’s” (Replacement, Reduction, Refinement)
- Interagency Research Animal Committee, *U.S. Government Principles for the Utilization and Care of Vertebrate Animal Used in Testing, Research, and Training* (1984)
- Animal Welfare Act and USDA Regulations
- Public Health Service Act and *PHS Policy on the Humane Care and Use of Laboratory Animals*
- *Guide for the Care and Use of Laboratory Animals*
- Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC)

The structure and function of the Institutional Animal Care and Use Committee (IACUC) is presented. Also discussed is utilization of services such as the National Agricultural Library and National Library of Medicine available to provide information on: 1) appropriate methods of animal care and use, 2) alternatives to the use of live animals in research, 3) prevention of unintended and unnecessary duplication of research involving animals.

“Level II Training” also provides special focus on the following topics:

- Consideration of alternatives, including the concept, availability, and use of research and testing methods that limit the use of animals, minimize the number of animals required to obtain valid results, and limit animal pain or distress
- Recognition and alleviation of animal pain and distress, including the proper use of anesthetics, analgesics, and tranquilizers
- Use of controlled substances and neuromuscular blocking agents
- Conduct of survival surgery, including proper pre-procedural and post-procedural care of animals, and aseptic surgical methods and procedures
- Euthanasia
- Development of humane endpoints
- Introduction to and instructions for enrollment in the Occupational Health and Safety Program

A summary of the IACUC’s Policies and Procedures manual is presented, with emphasis on “Responding to Reported Deficiencies in Animal Care and Treatment.” Various handbooks, guides, and other sources of information are distributed to seminar attendees, including copies of IACUC *Proposal* forms, OHSP materials, the UofL *OLAW Assurance of Compliance with the PHS Policy on the Humane Care and Use of Laboratory Animals* on file with the PHS Office of Laboratory Animal Welfare (OLAW).

All research personnel who work with animals must attend at least one Level II seminar within six months of their association with an animal research study. Completion of Level II Training is a prerequisite for access authorization to RRF animal facilities.

### **Level III:**

Special one-on-one or group training sessions provided by the RRF veterinary care staff or consultants (“Level III Training”) may also be recommended or required by the IACUC to ensure that research personnel are adequately prepared for a particular methodology. Additional didactic and/or wet-laboratory sessions are sponsored by the RRF and may also be attended on a voluntary basis.