POLICY

TITLE:	**Genotyping Rodents**
Policy Number:	**2014-028**
Responsible Department:	**Institutional Animal Care and Use Committee**
Policy Contact: **Designation:** **E-Mail:**	**Donald E. Walters, Ph.D.** **Chair, Institutional Animal Care and Use Committee** **dewalters@westernu.edu**
Approval Date:	**9/10/14**
Reviewed:	**8/9/17; 4/8/2020**
Revised:	**8/14/17 (Updated links); 4/8/2020 (Added reference to Survival Surgery policy for appropriate methods of sterilization)**

Purpose of Policy: To establish standards for genotyping rodents.

Policy Information: Proper genetic identification of genetically engineered animals is essential to facilitate research yet comply with the 3Rs in reducing the numbers of animals used in research. Although all of the following methods are acceptable, investigators should use the least invasive method when possible and consider them in the following order.

Fecal pellets are a non-invasive means of obtaining sufficient amounts of DNA for genotyping. The procedure does not require anesthesia and can be used on rodents of any age. Fresh stool pellets may be obtained directly from the animal or from its cage provided it is singly housed.

1. Broome RL, Feng L, Zhou Q, Smith A, Hahn N, Matsui SM, Omary MB. Non-invasive transgenic mouse genotyping using stool analysis. FEBS Letters 1999, 462:159-160.

2. Kalippke, K. DNA analysis from stool samples: a fast and reliable method avoiding invasive sampling methods in mouse models of bleeding disorders. Lab Animals 2009, 1-4.

Rectal swabs are a minimally invasive methods that can be performed on animals of any age. Anesthesia is not required.

1. Lahm H, Hoeflich A, Rieger N, Wanke R, Wolf E. (1998) Identification of transgenic mice by direct PCR analysis of lysates of epithelial cells obtained from the inner surface of the rectum. Transgenic Research, 131.4.

Buccal swabs are a minimally invasive method that can be used on rodents of any age. Anesthesia is not required.

Hair bulbs may be obtained by using forceps to pluck a tuft of hair. It is minimally invasive and does not require anesthesia.

1. Schmitteckert EM, Prokop CM, Hedrich HJ. DNA detection in hair of transgenic mice – a simple technique minimizing the distress on the animals. Lab Animal 1999, 33:385-9.

2. Cinelli P., et al. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. Lab Animals 2007; 41:174-184.

Blood – Obtaining blood is an invasive procedure that may require anesthesia depending on the method. Investigators are referred to the Institutional Animal Care and Use Committee's (IACUC) Policy 2014-004 on Blood Collection. However, a 2mm spot of dried blood on Whatman GF/C filter paper provides sufficient DNA for PCR.

1. Campbell DB, and EJ Hess. Rapid genotyping of mutant mice using dried blood spots for PCR analysis. Brain Research Protocols (1997) 1:117-123.

Tail biopsy is invasive and requires anesthesia for animals over 3 weeks of age, preferably with a short acting gas anesthetic like isoflurane. However, it is recommended that animals be less than 3 weeks of age because the yield of DNA is highest. Moreover, the tail ossifies between 2-4 weeks of age and ossified tissue yields less DNA per gram of tissue.

A maximum of 5 mm of tissue may be removed as larger samples do not yield proportionally larger amounts of DNA due to the presence of cartilage and bone that are not as rich in DNA as the distal end. Animals must be monitored to ensure effective hemostasis which can be accomplished either with pressure, cautery or styptic powder (e.g., Kwik Stop). However, electrocautery requires anesthesia. Resampling is not permitted without IACUC approval.

Regardless of age or species, sampling must be done with a sharp, sterile scalpel blade or scissors. The instruments must be thoroughly sterilized between animals to minimize infection and avoid DNA cross-contamination. Refer to IACUC Policy 2014-025 on survival surgery for appropriate methods of sterilization.

Investigators are cautioned that tissue removal from the distal tail may affect the results of certain behavioral tests (e.g. tail flick assay and hot plate response) later in life and that this should be taken into consideration when choosing this method.

1. Zhuo, Min. NMDA receptor-dependent long term hyperalgesia after tail amputation in mice. European Journal of Pharmacology 1998. Vol. 348, pp. 211-220.

Ear punches/notches are invasive procedures and for rats require anesthesia for animals over 3 weeks of age. Anesthesia is not required for mice regardless of age. A 2 mm ear punch is recommended as a lesser amount of tissue may not be sufficient for genotyping. The procedure may be performed at any age provided that the ears have separated from the head. A sharp punch must be used on the pinna (flap of the ear). Anesthesia or analgesia is not required, except for rats over three weeks of age, but the animal must be securely restrained.

Ear snipping may also be done in mice without anesthesia but anesthesia is required for rats older than 3 weeks of age. A 2-3 mm slice of the ear pinna is made using sharp scissors and thus avoiding ear blood vessels

Considerations: Animal body temperature and heart rate can transiently increase regardless of the method used.

Related Policy: IACUC Policy 2014-004, Blood Collection