

WVU IACUC Policy and Preferred Procedures: Rodent Genotyping and Identification

Purpose

To determine the genotype of genetically modified animals (GMA), it is necessary to obtain a tissue biopsy. Individual animal identification is important to this process to ensure accurate genotype records. Tissue harvest and identification is commonly performed before or at the time of weaning. The purpose of this policy is to establish standards for obtaining tissue while minimizing pain and distress to GMAs. Accurate genotype and identification records are necessary for design of appropriate breeding strategies and minimizing number of animals with unwanted genotypes.

The tail biopsy procedure is considered to be pain category C in animals ≤ 21 days of age and category D when appropriate anesthesia is used in animals > 21 days of age.

Policy

- A. Least invasive method that will produce accurate results should be implemented.
- B. If possible, tissue collection and identification should be combined into the same procedure (ear biopsy).
- C. Tissue collection should be performed at the youngest age which is reasonable for the study. This limits potential adverse outcomes.
- D. Important to minimize cross contamination between animal samples. This could result in inaccurate results.
 1. Start with sterilized instruments and clean with 70% ethanol between animals.
 2. Ensure work surface is cleaned with 70% ethanol.
 3. Samples should be immediately transferred into individually labeled collection tubes.
 4. Tissue collection procedures **must** be approved in the IACUC protocol.

Preferred Procedures for Mice

Veterinary staff can provide guidance on genotyping procedures in rats

- A. Pinna (ear) Biopsy
 1. Can be performed at any age, but is best in animals >14 days of age. The ear **must** be sufficiently developed for appropriate amount of tissue collection.
 2. Analgesia and anesthesia are not required for this procedure at any age.
 3. A 2 mm ear punch or notch is recommended.
 4. The procedure can be used as the primary identification method.
- B. Tail Biopsy
 1. Tail biopsy can be considered a potentially painful procedure and involves cutting through bone (if ossified), cartilage, blood vessels, soft tissue, and nerves. The amount of tissue excised should be the minimum amount needed.

2. This procedure should be performed as early as possible. The tail ossifies between 17-21 days of age. Ideally, this procedure is performed between 12-16 days of age before the distal portion of the tail is ossified.
3. Tail biopsy in adult animals causes signs of pain 1-2 hours post-procedure and thus requires local anesthetic use.
4. Procedure
 - a) Sterile sharp scissors, razor blade, or scalpel blade can be used to collect tissue.
 - b) Amount of tissue collected should be <5 mm.
 - c) Bleeding should be controlled with gentle pressure to incision site, styptic pencils, kwik stop, or tissue adhesive.
5. Considerations in mice ≤ 21 days of age
 - a) Pups should be kept away from dams for the least amount of time possible. Monitor pups closely after being placed back with the dam for signs of rejection.
 - b) Anesthesia and analgesia are not required if biopsy is performed at this age.
 - c) Local anesthesia (immersion of tail in 0.75% bupivacaine for 30 seconds post-biopsy) is recommended for animals between 17-21 days of age.
 - Animals in this age group should be monitored closely for signs of tail pain (tail grooming and twitching) post-procedure and local anesthetics implemented if observed.
6. Considerations in mice >21 days of age
 - a) General anesthesia and pre-emptive systemic analgesia are **required** when performing this procedure in animals over 21 days of age. Scientific justification is **required** if these treatments cannot be provided.
 - b) This procedure is not considered to be a surgery and sterile gloves/post-operative monitoring is not required.

C. Identification Methods

1. Ear Punch/Notch
 - a) Pups gently restrained via grasping loose skin on the dorsal neck.
 - b) Equipment used for this procedure should be cleaned with a high-level disinfectant prior to use and between animals. If the tissue collected will be used for genotyping, the instrument should be sterile before first use and cleaned with 70% ethanol between animals.
 - c) An ear punch instrument or sharp scissors should be used to puncture or cut ear tissue. The ear should be cut in the outer or middle region of the pinna. The area close to the head should be avoided.
2. Ear Tag
 - a) Ear tags should be placed using an ear tag instrument. Tools should be cleaned before use.
 - b) Ear tag is placed into the application pliers.
 - c) Locate fold of skin (area of pronounced cartilage) inside the rodent's ear.
 - d) Orient tag so numbers are visible at the top of the ear (dorsal surface)
 - e) Orient tag above the skin fold and squeeze pliers to apply the tag.
 - f) Avoid cartilaginous region of the lower ear.
 - g) Inspect ear to ensure proper placement and the ear is not folded or too close to ear edge.

3. Tattoo
 - a) Toe/Foot
 - Dip small gauge needle into commercially available tattoo ink. Apply small dots using needle to area which tattoo placement is desired.
 - Micro-tattoo systems are available for purchase.
 - b) Tail
 - Requires tattoo machine to be used.
 - Automated systems are available (Somark® Labstamp™ system).
4. Subcutaneous transponders
 - a) Follow manufacturer instructions for application.

Identification Method	Advantages	Disadvantages
Cage Card	<ul style="list-style-type: none"> • Identifies animal groups within the cage. 	<ul style="list-style-type: none"> • Can be lost or fall off cage. • Cannot identify individual animals.
Permanent Marker/Dye	<ul style="list-style-type: none"> • Used to write on tail or hair. • Non-invasive, low cost. • Easy to perform • Can be used in all ages, including young pups. 	<ul style="list-style-type: none"> • Marking is temporary and <i>must</i> be re-applied. • Can be groomed off easily.
Ear Punch/Notch	<ul style="list-style-type: none"> • Easy to perform, inexpensive • Can be done without anesthesia/analgesia. • Individual animals can be identified. • Tissue can be used for genotyping to limit number of procedures and reduce stress. 	<ul style="list-style-type: none"> • Recommended to be performed at 2 weeks of age or older. • Advanced numbering system can be difficult. • May become difficult to read over time. • Disrupted if animals tear or injure ear tissue.
Ear Tags	<ul style="list-style-type: none"> • Easy to perform, inexpensive • Can be done without anesthesia/analgesia. • Individual animals can be identified. 	<ul style="list-style-type: none"> • Recommended to be performed at 2 weeks of age or older. • Tag may rip out of ear. • Animals can have tissue reactions to ear tags. • Animals <i>must</i> be handled to read tags.
Tattoo foot/toe	<ul style="list-style-type: none"> • Can be done at any age without anesthesia/analgesia • Identification is permanent 	<ul style="list-style-type: none"> • May be difficult to perform. • Dye may impact draining lymph nodes.

Tail tattoo	<ul style="list-style-type: none"> • Can be done without anesthesia/analgesia • Identification is permanent • Extensive numeric identification system is possible. 	<ul style="list-style-type: none"> • Equipment can be expensive. • Can be difficult to read over time. • Cannot be used in small neonates. • Dye may impact draining lymph nodes.
Subcutaneous transponder (microchip, RFID tags)	<ul style="list-style-type: none"> • Some systems can be re-sterilized for re-use. • Extensive number system. Some microchips are writable and lab can assign individual ID. • Can be performed without anesthesia/analgesia. 	<ul style="list-style-type: none"> • Microchips can migrate. • Equipment can be expensive. Chip reader is required.

References

Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508186/pdf/jaalas2012000808.pdf>

Evaluation of Tail Biopsy Collection in Laboratory Mice (Mus musculus): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2687139/pdf/jaalas2008000010.pdf>