University Animal Care Committee Standard Operating Procedure		
Document No: **10.14**	**Subject:** **Genotyping Rats**	
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Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe standards for obtaining biopsy material for genotyping purposes while minimizing pain and distress to the rat.

1. **Introduction and Definitions:** To identify the genotype of a rat, tissue is biopsied to extract the genomic DNA. Tissue is harvested between 15-17 days of age to help minimize any pain or stress associated with the biopsy procedure. Ear notching (ear punch) or removing the most distal portion of the tail are the methods most often used. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes. Once the genotype has been determined, animal colony populations can be maintained at reduced animal numbers necessary for experimental efficiency. Genotyping should be completed before the animal is weaned at d21. This enables the user to cull or donate animals that are not of the genotype sought, and saves the generation of a cage card.
   - If tissue is required after 17 days of age, anaesthesia and analgesia are required (SOP 10.6 "Anesthesia in Rats").
   - Researchers should remove the least amount of tissue necessary to perform genotyping.
   - If animal identification is being performed through the removal of a piece of tissue, the same sample of removed tissue should be used for genotyping purposes.

2. **Materials:**
   - Ear notch instrument
   - Fine tipped forceps
   - Iris scissors
   - Sterile scalpel blade
   - Sterile gauze
   - 70% isopropyl alcohol or
   - Hydrogen peroxide or peracetic acid solution (HP-PA) or a
   - Bead sterilizer
   - Ruler
   - Anesthetics as required
   - Analgesics as required
   - Collection tubes
   - Styptic powder or silver nitrate sticks
   - Drapes or towels for restraint as required

3. Procedures:

Ear Punch Method

- The procedure is quick, easy, and should not cause bleeding if done properly. If bleeding does occur, apply gentle pressure with gauze until hemostasis occurs.
- This technique is best performed on 15-17d rats.
- Instruments used for ear notching can become dull after use and should be replaced often, as dull instruments can cause trauma to the notch site.
- Ensure the ear notch tool is clean of debris, and disinfected with alcohol or an HP-PA solution between each and every animal to prevent DNA contamination. Restrain animal using the scruff technique (as per SOP 10.20 "Manual Restraint of Rats").
- Place the device on the pinna of the ear (external ear) in the location wanted to mark the animal for identification.
- Press firmly to punch a circular hole through the ear (as indicated on the diagram below).
- As you remove the punch, be careful not to rip the delicate membrane of the pinna. If the punch does not remove all tissue, use forceps to gently hold the remaining skin tag and carefully cut free using iris scissors.
- Place the animal back in cage.
- Record animal's information on its respective cage card.
- For full circle numeration, ensure the hole is punched ~2mm in from the edge of the ear. This will prevent the hole from tearing, and possible misidentification with a semi-circle.
- If tissue from the ear biopsy is not completely removed, use forceps to isolate the remaining tissue and carefully cut the segment using iris scissors.
- Place animal back in cage.
- Record animal's information on its respective cage card.
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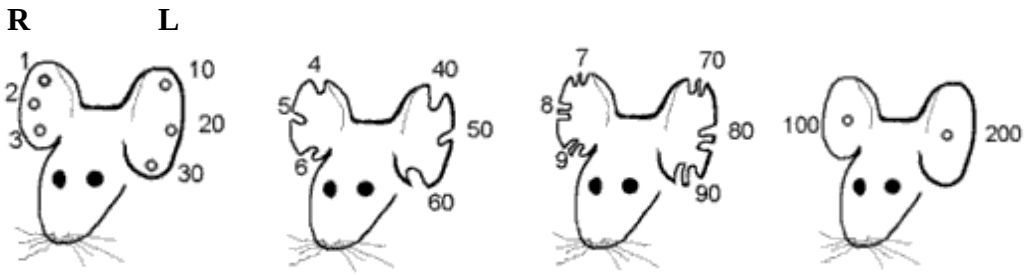

Distal Tail Snip Method

Recent studies have proven that tail snips can cause hypersensitivity even six months after the tail has been snipped. The last 5mm of the tail has tendons, nerves and coccygeal vertebrae that partly ossify by the age of 17 days. In a pre-weanling rat, the distal 2mm tail does not contain mature vertebrae (bone), thus, the tail biopsy should be performed at as young of an age as is feasible. With increasing age, tail maturation includes mineralization of bone and increased vascularity; it has been demonstrated that tail biopsy sampling performed on older rats can result in prolonged discomfort.

- General anesthesia and analgesia is required when tail biopsy is performed on animals older than 17 days of age.
- If general anesthesia has been administered fluid therapy must be provided, and the rat must be observed until it regains mobility.
- The maximum amount of tissue to be removed is one 4mm tail snip. The maximum number of tail snips that can be performed is one. If additional genotyping is required, an ear punch, fecal pellet or buccal swab must be used.
- Ensure the blade or scissors are clean of debris, and disinfected with alcohol or an HP-PA solution between each and every animal to prevent DNA contamination. Restrain animal using the scruff technique (as per SOP 10.20 “Manual Restraint of Rats”).
- Wipe the tail with 70% alcohol.
- Grasp the tail and referencing your ruler (laid flat), measure ≤4mm.
- Using either clean sharp iris scissors or a clean scalpel blade, cut the distal portion of the tail in one fluid motion.
- Any bleeding at the tail tip must be controlled following the amputation. Hemostasis can usually be achieved by direct manual pressure with gauze on the end of the tail. If direct pressure does not stop the bleeding, the use of hemostatic agents (e.g. Styptic powder such as KwikStop ®) is recommended and should be readily available as a precautionary measure.
- Return the animal to its home cage only once all signs of bleeding have stopped.

References:

1. Hankenson FC, Garzel LM, Fischer DD, Nolan B, Hankenson KD. Evaluation of Tail Biopsy Collection in Laboratory Mice (Mus musculus): Vertebral Ossification, DNA Quantity, and Acute Behavioral Responses. J Am Assoc Lab Anim Sci 2008; 47(6):10-18.

2. Pinkert CA. Transgenic Animal Technology: Alternatives in Genotyping and Phenotyping. Comp Med 2003; 53(2):126-139.

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