1.0 **Background:**

1.1 Individual animal identification is essential for the proper management of animals used in research and teaching at Washington State University (WSU). The procedures outlined in this SOP describe commonly used techniques for individual identification of small mammals, including rodents and rabbits. Enclosure-level identification of animals is acceptable in some circumstances.

2.0 **IACUC Requirements:**

2.1 All identification methods, including those outlined in this SOP, must be described and approved in the Animal Subject Approval Form (ASAF) before implementation. PIs may refer to this SOP within the ASAF for procedure details.

2.2 Personnel should be trained and initially supervised by someone who is proficient in the specific identification procedure prior to performing on their own. Training must be documented.

2.3 Animals should be monitored for appropriate recovery immediately following the identification procedure. All animals will be observed during the daily check.

3.0 **Method of Identification:**

3.1 **Identification cards - Species: Rodents and Rabbits**

3.1.1 Identification cards can be used at the room, rack, or cage level to identify groups or singly housed animals. Information on the card should include:

3.1.1.1 The source* and strain or stock of the animal(s)
3.1.1.2 Name(s) of responsible investigator(s)
3.1.1.3 The approved ASAF number
3.1.1.4 Pertinent dates as applicable, such as dates of birth, arrival, and surgery

*If the source for all animals in a room is the same it can be documented centrally (e.g., “in-house-bred” or in the animal’s medical record).

3.2 **Ear Punch or Notch - Species: Rodents**

3.2.1 Ear notches or punches can be used for the dual purpose of permanent animal identification and as a tissue sample for genotyping. Punches or notches should be placed in the periphery of the external ear (pinna), avoiding areas where cartilage is thicker close to the head. Tissue removed may be used for genotyping. Anesthesia is not required for this method of identification. A universal numbering system has been described (Figure 1)².

3.2.1.1 Use only sharp, commercially available ear punches
3.2.1.2 Disinfect the ear punch prior to use and between cages

3.2.1.3 Restrain the animal and notch/punch the ear(s) near or at the outer edge of the pinna

3.2.1.4 Collect only the minimum amount of tissue necessary

3.2.1.5 Control any bleeding with steady pressure to the site

3.3 Ear tags - Species: Rodents and Rabbits

3.3.1 Numbered, bar-coded or radio frequency identification (RFID) ear tags can be used as a method of permanent animal identification. Anesthesia is not required. Tag size and placement are important to increase retention and prevent tissue necrosis. Ear tags may cause tissue reactions and inflammation, which may require removal of the tag. Refer to tag manufacturer instructions for specific procedures. The following are general steps for ear tag placement:

3.3.1.1 Where possible, sterilize tags before use

3.3.1.2 Disinfect ear tag applicators prior to use and, at minimum, between cages of animals

3.3.1.3 Restrain the animal and place the tag in the lower third of the ear with the number/bar code facing forward (Figure 2)

Figure 1. Ear punch/notch code.

Figure 2. Restraint and proper placement of ear tags for rodents.
3.4 Microchipping - *Species: Rodents and Rabbits*

3.4.1 Microchips are easy to place, can provide nearly limitless individual alphanumeric identifiers, and rarely cause tissue reactions. A microchip reader is required to detect the alphanumeric code. Rodents may need to be sedated or anesthetized for proper microchip application. Animals should be large enough for the size of microchip used; it is recommended to perform the procedure after weaning. Please refer to manufacturer instructions for further guidance.

3.4.1.1 Sterilize microchips and trocars prior to use

3.4.1.2 Disinfect the skin at the site of application, usually between the shoulder blades

3.4.1.3 Tent the skin at the application site

3.4.1.4 Insert the trocar at the base of the skin tent and depress the plunger to insert the microchip subcutaneously

3.4.1.5 A drop of tissue glue may be used to seal the skin after implantation

3.5 Tattooing - *Species: Rodents and Rabbits*

3.5.1 An animal-grade tattoo gun, sterile small-gauge needle, or other appropriate device is used to create a permanent marking on the skin with tattoo ink. Pigment should be FDA-approved. Animals may be sedated or anesthetized for tattooing, although this is not required.

3.5.1.1 Disinfect the skin at the site of tattooing prior to introduction of ink. Tattoos are generally placed on the tail or feet of mice and rats, or the underside of the pinna of rabbits.

3.5.1.2 Use a sharp needle that is disinfected prior to use and at minimum between cages of animals. Needles should be replaced when dull.

3.5.1.3 Please refer to the tattoo device manufacturer instructions for device-specific ink products and procedures.

3.6 Toe Clipping - *Species: Rodents up to 7 days of age*

3.6.1 Toe clipping should only be used “when no other individual identification method is feasible” in mice or rats up to 7 days of age.³

3.6.2 Only the distal phalanx (last toe bone) may be removed, with no more than one toe per paw being clipped. It is preferable to remove toes from a hind paw rather than a forepaw, especially if the animals will be used in studies that include grip strength testing. If the forepaw must be used, it is preferable to not cut the hallux (“dew claw” or “little toe” of the forepaw). This method of identification must be scientifically justified and approved by the IACUC, and aseptic practices must be used. Tissue removed during toe clips may be used for genotyping. Anesthetics and analgesics should be used in accordance with animal age and species.

3.6.2.1 Use surgically sharp scissors. Scissors must be cleaned and disinfected beforehand and between each animal.
3.6.2.2 Aseptically prepare the digit before clipping
3.6.2.3 Apply pressure to the digit using a piece of gauze for several seconds
3.6.2.4 Monitor animals continuously until bleeding has stopped
3.6.2.5 Animals must be monitored to ensure appropriate healing of the toe

3.7 Non-Invasive Methods - Species: Rodents and Rabbits

3.7.1 Temporary, non-invasive mechanisms of identification include shaving or clipping of fur, non-toxic dyes, or permanent marker. These methods are short-lasting and may need to be re-applied within a matter of days. Only non-toxic markers, dyes, or pigments may be used.

4.0 References:

4.4 Guidelines for Toe Clipping of Rodents (nih.gov)