

Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE

INDIVIDUAL IDENTIFICATION TECHNIQUES

Purpose: It is oftentimes necessary to distinguish individual animals from each other. In some cases, natural traits such as size, color, or shape can be used as unique identifiers, but more commonly individuals are not easily recognizable. In such cases, it is required to use a technique that identifies them individually. There are numerous options for identifying research animals, and choosing which method is best depends on the particular situation. Common factors used to pick an identification method include:

- Species to be marked
- Duration the identifying mark has to last
- Whether the mark needs to be unique to all animals or just among cagemates
- Invasiveness of the procedure
- Ease of application
- Ease of reading

Below are descriptions of the more common identifying techniques used in research:

Indelible ink (e.g., Sharpie pen)

Species: any animal that has bare skin, particularly rodent tails or ears, lizards, or snakes

Pros: Inexpensive; easy to apply; easy to read without picking animal up

Cons: not permanent (3-4 days for rodents, typically until their next shed for reptiles); can be difficult to include details (e.g., ID numbers if writing on a tail)

Description: Simply restrain the awake animal as appropriate for the species and location where mark is to be applied, and then write the desired mark on the animal's skin. Check regularly and re-apply as needed to avoid losing mark completely.

Animal marker

(e.g., <https://www.stoeltingco.com/animal-markers.html#:~:text=Unlike%20permanent%20markers%20and%20art,in%20food%2C%20drugs%20and%20cosmetics.&text=Color%20will%20not%20remain%20on%20the%20animal's%20skin>)

Species: animals with light-colored hair or feathers

Pros: easy; relatively long-lasting

Cons: can permanently stain surrounding area when being applied

Description: An awake animal is restrained by hand and a mark is applied. This mark generally lasts 2-3 months and can be darkened by reapplication if it fades over time.

Tattoo

Species: any animal where skin is not covered by hair or feathers

Pros: permanent; can provide details (e.g., sequence of numbers)

Cons: requires special equipment to apply; time-consuming to apply; hand-tattooing requires considerable practice; may require handling of animal to read tattoo; may be difficult to read if hair grows over it.

Description:

Anesthesia may or may not be required based on the species and the skill of the person applying the tattoo. There are several types of handheld micro-tattooing equipment. Training should be obtained on the specific micro-tattooing system that is to be used. It is important to prevent potential cross contamination associated with the use of this equipment. An automated tattooing unit (Labstamp) is commercially available.



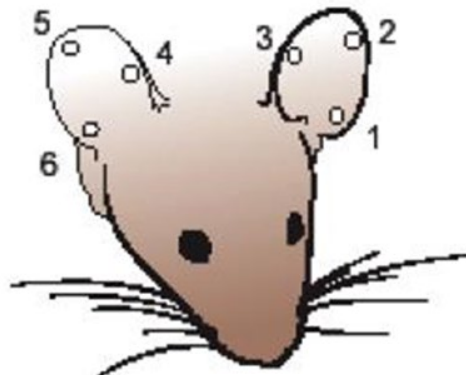
Ear punch or notch

Species: rodents including chinchillas

Pros: permanent (but secondary damage can alter readability); can typically read without picking the animal up, provides a source of tissue for genotyping

Cons: moderately invasive; not the preferred method in immune compromised animals

Description: Different sized ear punches are available. Ear punches should be disinfected (e.g., with 70% ethanol or chlorhexidine) prior to use and between each animal. Make sure that the ear punch is sufficiently sharp prior to use; having a spare is a good idea. Punches can be simply 1-2 punches in either ear to distinguish animals within a cage or a combination of punches according to a numbering scheme to separately identify larger groups of animals (see figure). To identify a mouse by ear punch, restrain it by the scruff and then position the punch approximately 3 mm from the edge of the ear pinna. A notch, rather than a punch can be created by performing the punch at the edge of the ear, but notches can be more difficult to read than punches. Care should be taken to not place the punch too far towards the base of the ear; doing so can injure the animal's ear and makes reading the punch more difficult. Larger animals (e.g., rats, chinchillas) can be ear-punched with a large punch using a restraint technique that is appropriate for the species.



Sample ear punch number scheme



Ear-punching using a rodent ear punch

Ear tag

Species: rodents; rabbits

Pros: Permanent unless it comes off as a result of poor application technique or persistent scratching at it by the animal.

Cons: application induces some pain; bulky; can cause significant local irritation and even infection requiring the tag be removed; difficult to read number without picking the animal up

Description: It is best to rinse the tag with 70% alcohol and then let it dry before applying it to the ear. For metal clip tags, the most common ones currently used (see image below), place the tag in the applicator so that the holed end of the tag is positioned over the notched area of the applicator. The pointed end should be opposite the hole. Scruff the mouse so that the ear is easily accessible. Place the ear between the point and the hole of the tag. The numbers should face upward so that they can be easily read without restraining the animal. The tag should be positioned at the lateral base of the ear, approximately 3 mm from the edge of the ear pinna (see images below). Once the tag is positioned correctly, squeeze the applicator firmly to apply the tag. For other types of tags, the process is similar, but they use different applicators (see images below for examples). For all tags, monitor the tag implantation intermittently for signs of local infection or inflammation (redness, swelling, bleeding). If such signs are detected, the tag should be removed; a new tag can be placed in the other ear if needed. Additionally, tags may be placed on one side for males and the other side for females to allow quick first-level identification of breeding pairs.



Toe clipping

Species: juvenile rodents; small lizards

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Pros: permanent (but secondary damage can alter readability); provides a source of tissue for genotyping

Cons: considered highly invasive (need to justify why other methods cannot be used); difficult to read without picking the animal up

Description: Mice to be toe-clipped should be under 17 days of age. Toe-clipping of mice older than 17 days requires scientific justification and must be specifically reviewed by the IACUC. No more than 2 digits (total) can be clipped, and they must be on separate limbs. The digit can be severed at any level distal to the middle of the 1st phalanx. Use clean, sharp scissors or a clean, sharp scalpel blade to remove the toe segment. Between animals, the scissors or blade should be either bead sterilized or cleaned with an antiseptic solution (e.g., isopropyl alcohol, 70% ethanol, or chlorhexidine).

According to the Wildlife Society and other wildlife specialists, toe clipping is the most common method for permanently marking lizards (Mietfeld et al., 1994, Waichman 1992) and has been found to not affect short-term recapture rate in some lizards (Jones and Bell 2010). Toe-clipping should only be used in small (e.g., < 40 g) lizards. Furthermore, toe-clipping is actually less stressful than the technique frequently recommended to replace it (microchip implantation) and even less stressful than superficially trivial manipulations such as housing the animal in an unfamiliar enclosure (Langkilde and Shine, 2006). For identification, a code for the toe clips should be used, the complexity depending on the number of individuals that need to be marked (e.g., Waichman, 1992, where they assign letters to the feet and numbers to the toes). Lizards should be held firmly but gently in the non-dominant hand of the handler so that the foot of the toe being clipped is immobilized between two fingers. Using sharp scissors, the toe is clipped near the knuckle, but not into the knuckle. If bleeding occurs, pressure should be applied between two fingers until the bleeding stops. Styptic powder can be used to control bleeding, but this typically is unnecessary. Lidocaine (topical gel) should be applied to the wound immediately after cutting the toe. Scissors should be placed in a bead sterilizer or wiped with 70% alcohol or chlorhexidine between animals.

References:

- Jones, C., and T. Bell. 2010. Relative effects of toe-clipping and pen-marking on short-term recapture probability of McCann's skinks (*Oligosoma maccanni*). *Herpetological Journal* 20: 237-241.
- Langkilde, T., and R. Shine. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. *Journal of Experimental Biology* 209: 1035-1043.
- Nietfeld, M. T., M. W. Barrett, and N. Silvy. 1994. Wildlife marking techniques. In Bookhout, T. A. *Research and Management Techniques for Wildlife and Habitats*. P. 140-168. Wildlife Society, Bethesda, MD.
- Waichman, A.V. 1992. An alphanumeric code for toe-clipping amphibians and reptiles. *Herpetological Review* 23: 19-21.

Leg banding

Species: birds

Pros: permanent; standard for wild birds, often required by regulatory agencies, easy to read if based on color

Cons: Cannot read numbers without picking the animal up; can cause injury if too small a band

Description: Make sure to choose an appropriately sized band. For plastic bands, simply unroll the coil and then place it around the leg. It will re-coil once you let go. For metal leg bands, Place the opened band around the bird's leg. Surround the band with the appropriately sized notch of the applicator, and squeeze the applicator shut to correctly seal the band. If the band does not fully

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seal on the first squeeze of the applicator, rotate the band in the applicator and squeeze the applicator again. Continue rotating the band and squeezing the applicator until it is completely sealed. If bands need to be removed, this must be done very carefully; creating torque on the leg can easily fracture the leg.



Passive Integrated Transponder microchips (i.e., PIT tags)

Species: any

Pros: permanent; number is globally unique; some PIT tags also provide temperature when read

Cons: relatively expensive; impossible to read without having a reader (can be inconvenient)

Description: PIT tags come in various sizes. They are inserted using special syringe-like applicators (see image below). Anesthesia is not required but can make the process easier in some species. Test the PIT tag before implanting it into the animal by scanning it with a compatible reader while it is still in the package (do not contaminate it during this process). It is advantageous to shave or part by wetting any hair (or pluck or part any feathers) over the area to be implanted. Apply disinfectant (e.g., chlorhexidine) to the area. For mammals, PIT tags are typically implanted by making a tent from the loose skin over the shoulder blades, inserting the needle subcutaneously with the bevel up, and then depressing the plunger (see image below). For reptiles and fish, PIT tags can be implanted intracoelomically (small individuals) or into the epaxial muscles (larger individuals). Once the applicator needle is removed, the injection site should be observed for bleeding. If bleeding is noted, digital pressure with a gauze pad should be applied. Typically, the insertion site does not need to be closed, but a drop of surgical glue can be applied if desired. Once implanted, test the PIT tag again to confirm proper function. PIT tags can be reused only after proper cleaning and sterilization (following manufacturer's recommendation).

