

# Tattooing Various Combinations of Ears, Tail, and Toes to Identify Mice Reliably and Permanently

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Uniquely identifying research animals is a widespread and essential procedure. Potential disadvantages of commonly used identification methods such as toe clipping, ear punching, and ear tagging include tissue loss and adverse effects in physiologic homeostasis and animal behavior. In addition, the labels produced by using these methods can become unreadable, potentially leading to misidentification. In this study, we proposed a combined approach involving ear, tail, and toe tattooing that can be used to permanently identify mice regardless of their age. Four groups (neonatal and adult C57BL/6J [black] and CD1 [white] mice) were used. Single- or 2-color tattooing (ear, tail, or toe or combinations thereof) was performed to identify a defined or unlimited number of mice, respectively. Tail tattooing using both green and red pastes was suitable for identifying white-haired neonatal mice as early as postnatal day 1, whereas toe tattooing with green paste was an effective alternative approach for labeling black-haired mouse pups. In comparison, single-color (green) or 2-color (green and red) ear tattooing identified both white and black adult mice older than 3 wk. Ear tattooing can be adapted to labeling an unlimited number of adult mice by adding the cage number. We conclude that tattooing various combinations of the ears, tail, and toes provides an easy and permanent approach for identifying mice of all ages with minimal disturbance to the animals.

Laboratory mice are one of the most commonly used animal models in the biologic and biomedical fields. Because mice usually are housed in groups, uniquely identifying individual mice becomes an essential procedure for all laboratories to accurately record a mouse's information into the project's database. This database may include unique properties of the experimental or control subjects, the origin of tissue or cells, phenotype and genotype information, breeding information, colony management, health records, and other related functional and biologic measurements.<sup>2</sup> However, many factors should be considered when selecting the identification method so that it fits the type of mice and the needs of the project, such as the availability of identification methods and tattooing paste, mouse strain and age, skin pigmentation and hair color, potential side effects of the identification method on physiologic functions, as well as regulatory and policy requirements for mouse housing and breeding within each institute. Therefore, the ideal identification method is not only easy and efficient but also minimizes any associated pain and distress.<sup>2</sup>

Several classic methods often used to label mice include ear tagging,<sup>3</sup> ear punching or notching,<sup>4</sup> tail tattooing,<sup>11</sup> toe clipping,<sup>2,7</sup> toe tattooing,<sup>8</sup> and implanting microchips (transponders),<sup>4</sup> with each method having its advantages and disadvantages (Figure 1). For example, methods involving the removal of tissue (for example, toe-clipping, ear-punching or -notching) are easy, simple, and perhaps the most frequently used methods in both neonatal and adult mice.<sup>2,7</sup> One major advantage of these approaches is that the collected tissue can be used for genotyping transgenic mice. However, the main drawbacks of these methods, especially of toe clipping, involve their adverse effects on various physiologic parameters, animal behaviors,<sup>9</sup> and the possibility of acute or chronic infection.<sup>3</sup> Therefore, ethical concerns and issues regarding animal well-being after the use of these methods have been raised at many

research institutes. In addition, mislabeling and misidentification of animals because of unreadable marks often occur due to the regrowth of excised tissue. Although toe tattooing can be used as an alternative approach to minimize distress and tissue damage to neonatal and adult mice,<sup>2,8</sup> this method can be associated with toe infection, bleeding, and mislabeling (especially in neonatal mice). The high-tech microchip-based method (for example, radiofrequency transponder<sup>4</sup>) uses a wireless transponder, which is implanted subcutaneously into a mouse and can be read without touching or disturbing the animal. This method, however, is cost prohibitive for many laboratories.<sup>2</sup> Therefore, an ideal identification method would provide easy, efficient, and permanent identification without generating significant pain or distress and remains an urgent need for mouse research laboratories.

In the present study, we developed a new combined ear-, toe-, and tail-tattooing approach, using C57BL/6J (black) and CD1 (white) mice as example strains, to identify mice ranging in age from neonate to adult. Two-color (green and red) tail tattooing can be used to identify white mouse pups as early as postnatal day 1 (PND1), whereas classic 1-color toe tattooing can be used instead of toe clipping to identify black mouse pups (currently, no tattooing paste or ink suitable for use in black mice is commercially available). In contrast, single- or 2-color ear tattooing can be used to mark adult mice older than 3 wk, regardless of skin or hair color. By adding the cage number (on the tail or ear) to the mouse identification number, an unlimited number of mice can be labeled uniquely and permanently.

## Materials and Methods

**Animals.** Both female and male black (C57BL/6J) and white (CD1) mice, originally purchased from Jackson Laboratory (Bar Harbor, ME) or obtained from our colleague (Dr Michelle Theus) at 8 to 10 wk of age, were used as the breeding pairs for the present study. After birth, pups stayed with their parents in the same cage until PND21, when the pups were weaned and randomly housed as single-sex groups in solid polycarbonate cages (30 cm × 20 cm × 15 cm). A maximum of 5 mice per cage

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Method	Suitable mice	Major advantages	Major disadvantages
Ear tattooing (this study)	• Adult mice (all colors)	<ul style="list-style-type: none"> <li>• Easy to perform</li> <li>• Labeling can be permanent and is easily read</li> <li>• Unlikely to have bleeding and infection</li> <li>• Large numbers of mice can be labeled when 2 colors and cage number are used</li> <li>• Effective for both white and black mice</li> <li>• Various colors of tattooing pastes can be used, depending on color of skin or hair</li> <li>• Tattooing can be digitized by using a special device (for example, NEO-9 system [AIMS, Hornell, NY])</li> </ul>	<ul style="list-style-type: none"> <li>• Excess ink on needle may result in mess or contamination of targeted spots on the ear</li> <li>• Unsuitable when genotyping uses ear tissue</li> <li>• A limited number of marks can be made on each ear</li> <li>• Nonconsecutive identification numbers</li> <li>• Unable to use on neonatal mice</li> </ul>
Ear tagging	• Adult mice (all colors)	<ul style="list-style-type: none"> <li>• Classic method and easy to perform</li> <li>• Labeling can be permanent and is easily read</li> <li>• Large numbers of mice can be easily labeled with preprinted tag</li> <li>• Can be digitized using a special device</li> </ul>	<ul style="list-style-type: none"> <li>• Potential for bleeding and infection</li> <li>• Tag is easily lost from the ear and therefore may cause misidentification</li> <li>• Ear can be damaged</li> <li>• Heavy metal tag makes mice uncomfortable</li> </ul>
Ear punching or notching	• Adult mice (all colors)	<ul style="list-style-type: none"> <li>• Classic method and easy to perform</li> <li>• Labeling can be permanent</li> <li>• Large numbers of mice can be easily labeled by using various combinations in both ears</li> <li>• Removed tissue can be used for genotyping</li> </ul>	<ul style="list-style-type: none"> <li>• Potential for bleeding and infection</li> <li>• Can be difficult to read when holes are too close together or regrow</li> <li>• Tissue healing can cause miscounts or make punches hard to read</li> </ul>
Tail tattooing (this study)	<ul style="list-style-type: none"> <li>• Neonatal mice (white)</li> <li>• Adult mice (white)</li> </ul>	<ul style="list-style-type: none"> <li>• Labeling is clear on white mice before weaning (ear-tattooing method is suggested after weaning)</li> <li>• Various colors of tattooing paste can be used, depending on the color of skin or hair</li> <li>• Can be digitized by using a special device</li> </ul>	<ul style="list-style-type: none"> <li>• Potential bleeding, infection, and tail damage</li> <li>• Unsuitable when tail tissue is needed for genotyping studies</li> <li>• Pastes or inks suitable for use in animals are not available commercially</li> <li>• Limited number of marks can be made on tail</li> <li>• Poor labeling on black mice</li> <li>• Limited number of mice can be identified when cage number is omitted</li> </ul>
Toe clipping	<ul style="list-style-type: none"> <li>• Neonatal mice (all colors)</li> <li>• Adult mice (all colors)</li> </ul>	<ul style="list-style-type: none"> <li>• Classic method and easy to perform</li> <li>• Clipped toe tissues can be used for genotyping</li> <li>• Labeling can be permanent and is easily read, especially in adult mice</li> </ul>	<ul style="list-style-type: none"> <li>• Potential bleeding, infection, and damage to foot tissue</li> <li>• Toes may grow back if clipping is done in neonatal mice, thus complicating reading</li> <li>• Unsuitable for behavior or movement studies, which require intact toes and feet</li> <li>• Tissue healing may cause miscounts or make clips hard to read</li> <li>• Limited number of toes can be clipped</li> </ul>
Toe tattooing	<ul style="list-style-type: none"> <li>• Neonatal mice (all colors)</li> <li>• Adult mice (all colors)</li> </ul>	<ul style="list-style-type: none"> <li>• Classic method, with minimal tissue damage</li> <li>• Mark is permanent and easily read</li> <li>• Numerous numerical combinations make it possible to label a large number of mice</li> <li>• Various colors of tattooing paste can be used, depending on the color of the skin or hair</li> </ul>	<ul style="list-style-type: none"> <li>• Potential for bleeding and infection</li> <li>• Potential undesired marking of adjacent digits, resulting in a miscount</li> <li>• Somewhat challenging when performed on early-neonatal mice</li> <li>• Excess ink on needle may result in mess or contamination when targeting spots on toes</li> </ul>
Microchipping	<ul style="list-style-type: none"> <li>• Neonatal mice</li> <li>• Adult mice</li> </ul>	<ul style="list-style-type: none"> <li>• Accurate, unique identification for neonatal (older than 10 d) and adult mice</li> <li>• Unlimited identification</li> <li>• Wireless signal transponder</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive</li> <li>• Infection and other side-effects</li> <li>• Not suitable for mice younger than 10 d</li> </ul>

**Figure 1.** Comparison of common identification methods in mice.

(weaned or adult) were housed together according to Virginia Polytechnic Institute and State University's IACUC policy. Animal staff changed cages, bedding, water bottles, and aspen shelters weekly. The ambient temperature of the animal facility was 22 to 23 °C, and the relative air humidity was kept at 50% to 70%. Room illumination followed a 12:12-h light:dark cycle (lights on, 0700). Mice had free access to food and tap water.

At least 3 mice in each group (black compared with white and neonatal compared with adult) were used in this study to examine 2 new identification methods (tail and ear tattooing). A total

of 12 adults (age, 3 wk to 5 mo) and 12 pups (PND 1 through 5) of both black and white mice were used. Because toe tattooing has been widely used and because the literature contains various coding methods to label mice by using all 4 feet,<sup>7,8</sup> we only briefly present our modified coding systems using the 2 front feet. All procedures conducted in this study were approved by the IACUC at Virginia Polytechnic Institute and State University.

**Tattooing tools and pastes.** Currently, only 2 colors (green and black) of animal tattooing pastes are available commercially (Ketchum Manufacturing, Lake Luzerne, NY). Therefore, we

purchased human tattooing inks (white, red, yellow, and blue) from Hennaking (Las Vegas, NV) as replacements to test tattoo visibility on black and white mice. Fine scissors (catalog no. 14060-10, Fine Science Tools, Foster City, CA), a glass-bead dry sterilizer (Stoelting, Wood Dale, IL), and an anesthetic system (EZ-7000, Euthanex, Palmer, PA) were purchased as indicated. Other common reagents and supplies, including alcohol wipes or gauze, cover-glass forceps, scissors, 27- to 30-gauge needles, and 1-mL syringes (Figure 2 A), were purchased from Thermo Fisher Scientific (Waltham, MA) unless otherwise stated. A 12.1-megapixel camera (PowerShot SX50 HS, Canon, Tokyo, Japan) was used to take images of the tail and toe tattoos, and an 8.0-megapixel camera (OMAX, South Kent, WA), which was installed on a stereomicroscope (SZ6.1, Olympus, ), was used to take images of the ear tattoos.

**Identification methods.** All procedures were performed under anesthesia, as required by our IACUC. Briefly, a neonatal or adult mouse was placed in an induction chamber and initially supplied with 3% to 5% isoflurane for 2 to 3 min and then maintained under 1% to 2% (adult mice) or 2% to 4% (neonatal mice) isoflurane during the identification procedures. Mice did not need to be physically restrained nor was a heating-pad necessary because each procedure only took a few minutes to complete. The pain induced by the tattooing procedures is supposed to be transient and minimal, so anesthesia may not be necessary or required by IACUC at other institutes. However, we suggest the application of easily used isoflurane to reduce potential pain.

**Tail tattooing procedure and the 2-color numbering system.** Under the anesthetic conditions described earlier, a mouse pup was held gently by its lower body, its tail wiped with a 70% alcohol pad, and its tail tattooed according to the procedure described previously.<sup>11</sup> Briefly, the tip of a 27- to 30-gauge hypodermic needle attached to a 1-mL syringe was lightly dipped into the tattooing paste and subcutaneously inserted into the central line of the dorsal tail of a neonatal mouse at about a 45° angle. This procedure can be performed on any day after birth, but PND7 or earlier is preferred, before the tail hair grows too dense. After being tattooed, the tail was gently wiped with dry gauze to remove any excess paste, and the mouse was returned to its home cage. Needles were cleaned with a 70% alcohol pad or sterilized in a glass-bead sterilizer after use, a new needle was used for each mouse, and the sterilized needle was allowed to cool down before use. These aseptic techniques were applied to the other labeling methods described following. The entire procedure only takes 1 to 3 min for anesthetized mice, after minimal practice (Figure 2 B).

Mice usually give birth to litters of 3 to 14 pups with an average of 6 to 8, and pups are not weaned until 3 wk after birth. Therefore, to identify individual neonatal pups, which might be required in some studies, we conducted the tail-tattooing procedure by using a 2-color numbering code (Figure 3), in which each green dot represented a value of 1, and each red dot represented a value of 5; the combination of both colors made it possible to uniquely identify a total of 20 pups, which is larger than the usual size of litters. In our preliminary test, we examined various available colors of both animal and human tattooing pastes and inks and found that the green and red pastes and inks were best suited to white mice (Figure 3 A). White, yellow, and blue inks (all human tattooing inks) did not maintain strong marks on the tails of black mice; we consider that this result was mainly due to the watery consistency of these products. We therefore used the toe-tattooing method as an alternative approach to identify black mouse pups in the present study, and we will test other colors of animal tattoo pastes once they are available commercially.

**Toe-tattooing procedure and the numbering system using the 2 front feet.** Toe tattooing can be performed on neonatal mice.<sup>2</sup>

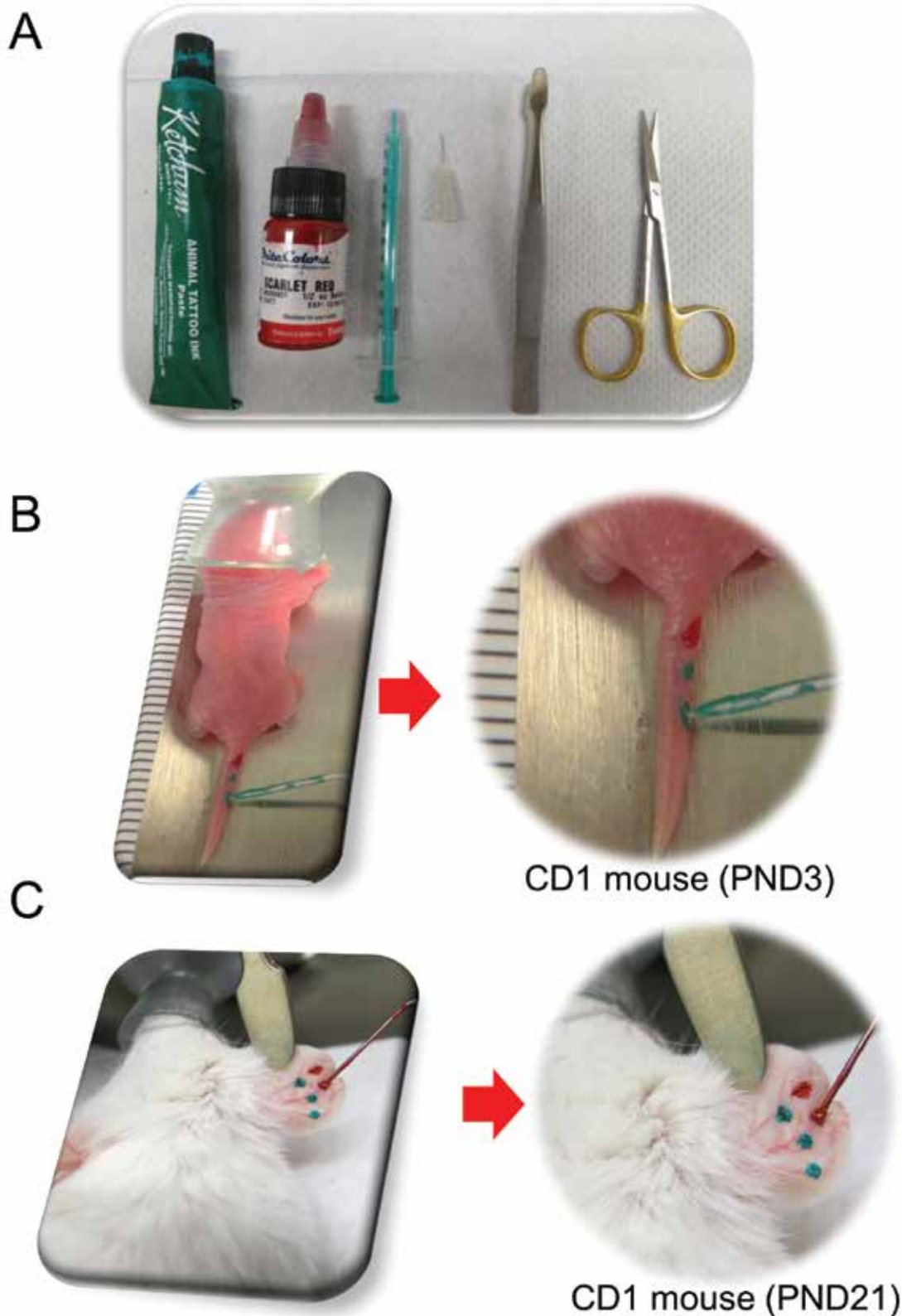
In published studies, the classic toe-tattooing methods use a 4-feet numbering system, so the mice experience pain (albeit minimal) in all of their feet. In the present study, we suggest a simple coding system that targeted the 2 front feet only, not only to minimize stress to the animals but also to keep the rear feet untouched, as might be required in some projects (for example, studies involving hindlimb ischemia). Using both front feet, we were able to uniquely identify 1 to 14 mouse pups (Figure 3 B). The toe-tattooing procedure we used was almost the same as those described for tail and ear tattooing (see the earlier and following sections and Figure 2 B). The key step was that the needle must penetrate through each toe.

**Ear-tattooing procedure and the single- and 2-color numbering systems.** For ear tattooing, adult mice were anesthetized and prepared aseptically as for the tail-tattooing procedure mentioned above. A 27- to 30-gauge needle that had been dipped in tattoo paste (green or red), was used to puncture 1 to 10 holes in a 'C' shape on one or both ears of a mouse (Figures 2 C, 4, and 5). The dye-containing tip of the needle was pushed through the ear from the outside at close to a 90° angle while the ear was held gently with cover-slip forceps. The needle was held in the site for 1 to 2 s to ensure staining before removal. It is important not to dip too much ink on the tip of the needle, to prevent the excess from contaminating the neighboring area. However, excess paste can easily be removed by using a cotton swab or dry gauze.

Ear tattooing with a single color of paste has been previously reported in rats.<sup>6</sup> We propose 2 different numbering systems for ear tattooing mice by using either a single- or a 2-color approach. In the single-color numbering system, 1 to 5 pierces (green dots) on the right ear were made to indicate a mouse identification number of 1 to 5, whereas the green dot(s) on the left ear represented the transfer code for mice that were transferred into the current cage from a previous one (Figure 4). When a nonrepeatable cage number was combined with the mouse number, the total number of mice could be labeled infinitely (for example, 'C4 no. 5' to mean 'cage 4 mouse no. 5,' Figure 4 B). This method could be applied for adult mice with any coat color. In contrast, the 2-color numbering system, in which 1 to 5 pierces per color per ear can be generated (inner loop of green and outer loop of red, Figures 2 C and 5), is designed to identify individual mice without adding the cage number. In this system, 4 digits from  $10^3$  to  $10^0$  (read from right to left ear and from the outer to the inner loop) represents the 1000's place ( $10^3$ , red on the right ear), 100's place ( $10^2$ , green on the right ear), 10's place ( $10^1$ , red on the left ear), and the 1's place ( $10^0$ , green on the left ear; Figure 5). Thus numbers between 1 and 5555 can be generated. Unfortunately, the entire range of potential identification numbers (1 to 5555) is nonconsecutive, with various numbers missing due to the quinary numbering system used in this study. The described system actually yields a total of only 1295 unique identifiers when cage number is not included.

## Results

**Using 2-color tail tattooing to identify white mouse pups.** To label individual pups within a harem breeding cage, we proposed a 2-color tail-tattooing system to uniquely identify mouse pups to a maximum of 20 in a single cage (Figure 3 A). Green and red pastes generated clear tattoo marks on the tails of white-haired mouse pups; white, yellow, and blue pastes failed to do so on black-haired mouse pups (data not shown). Therefore, we used the green and red pastes on the tails of white mouse pups, instead opting for toe tattooing an alternative method for identifying black mouse pups. Figure 3 A shows the tattoo marks on the tail of a white mouse on PND3 (left) and then at

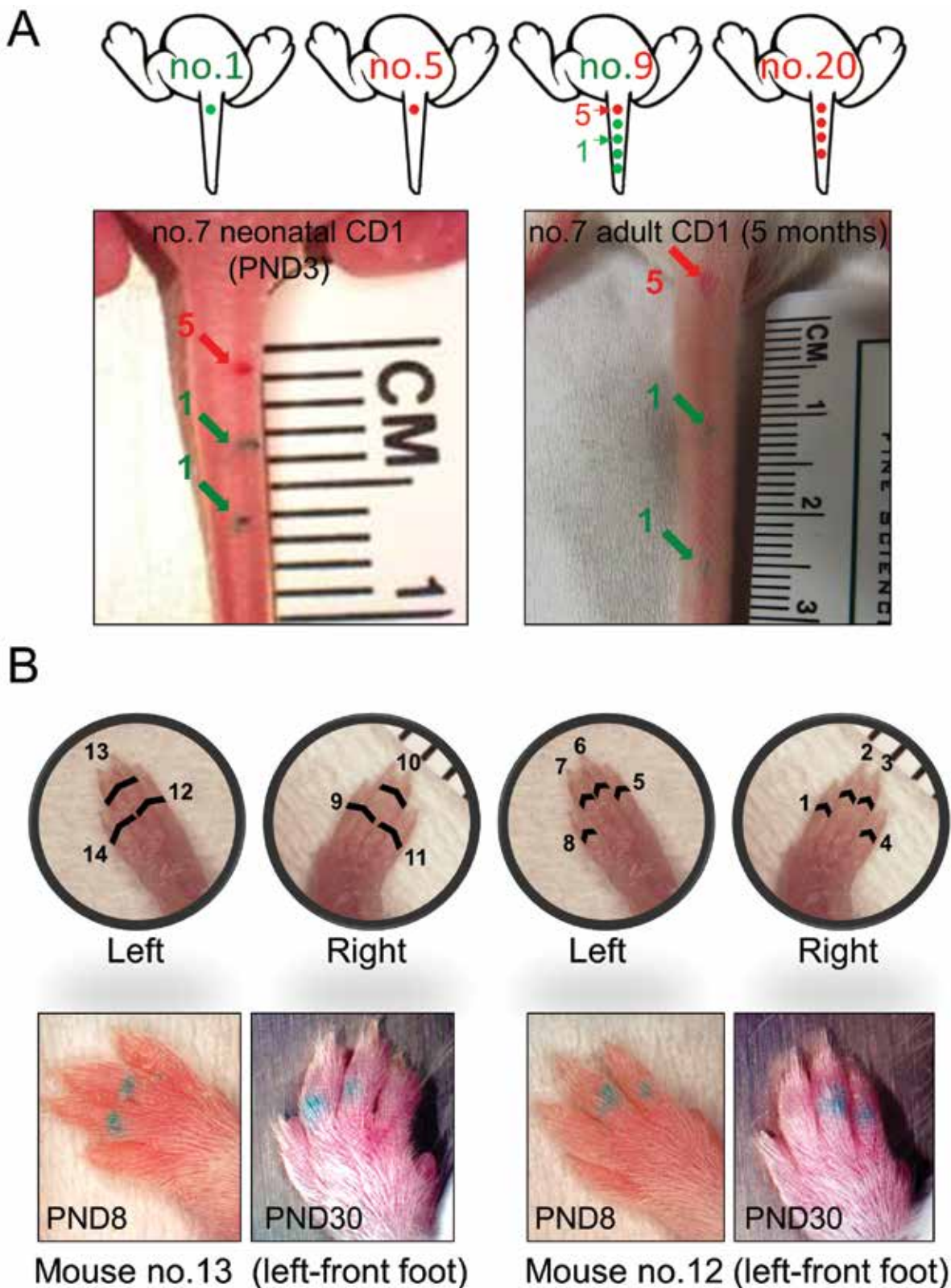


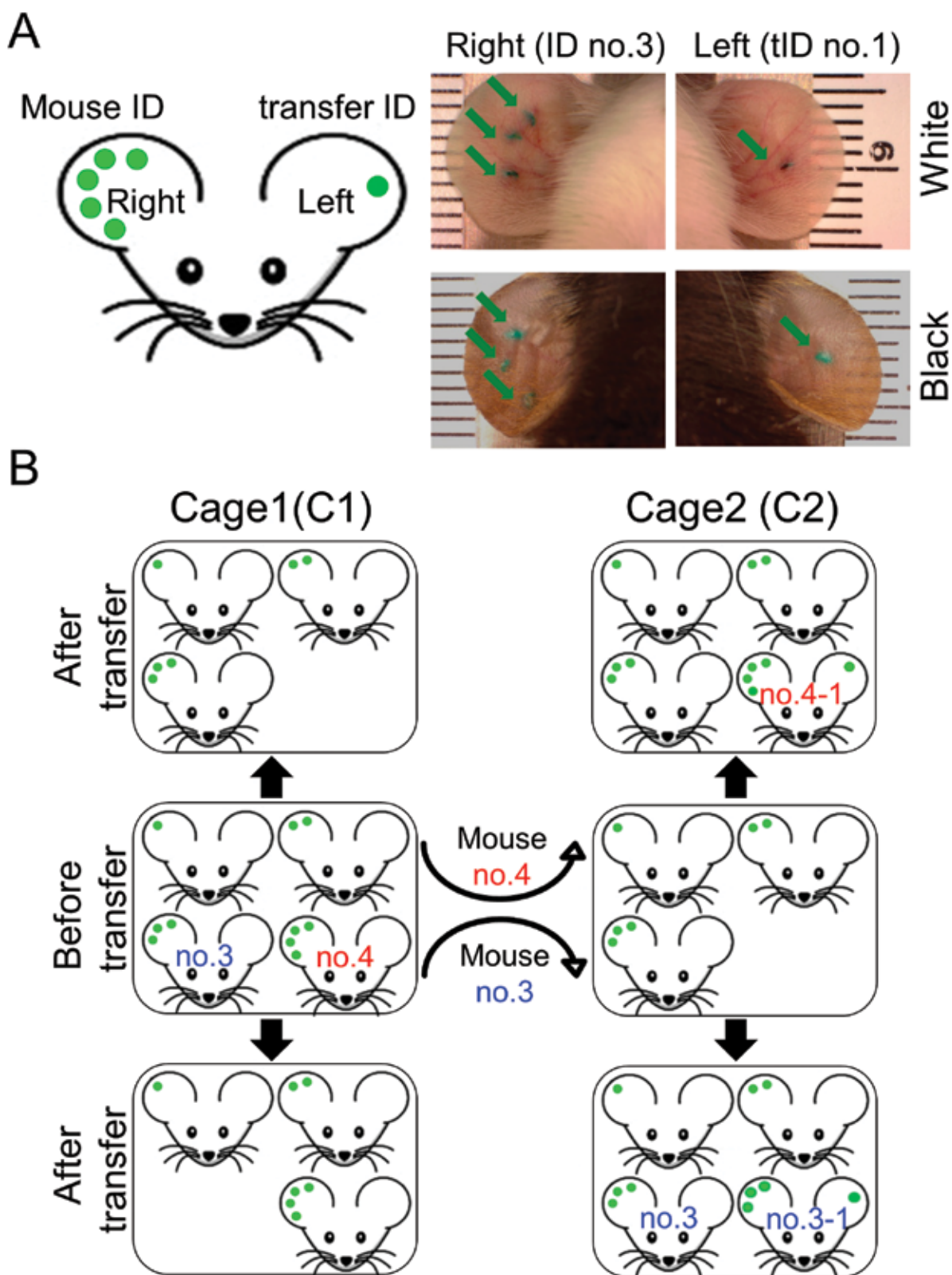
**Figure 2.** Mouse tattooing materials, tools, and procedures. (A) Tattooing material and tools used for tattooing the ears, tails, and toes of mice, including tattooing ink (green and red), 1-mL syringe, 27- to 30-gauge needle, cover-glass forceps, and iris scissors. (B) Tail-tattooing procedure and representative marks (1 red and 2 green dots) on the dorsal tail of a neonatal CD1 (white) mouse on postnatal day (PND) 3. (C) Ear-tattooing procedure and representative marks (3 green and 2 red dots) on the right ear of adult CD1 (white) mouse.

5 mo after tattooing (right). In this example, mouse no. 7 was labeled with 1 red dot (a value of 5) and 2 green dots (total value, 2). The tattoos could still be seen by naked eye even after the tail hair had grown in fully. We used this method to

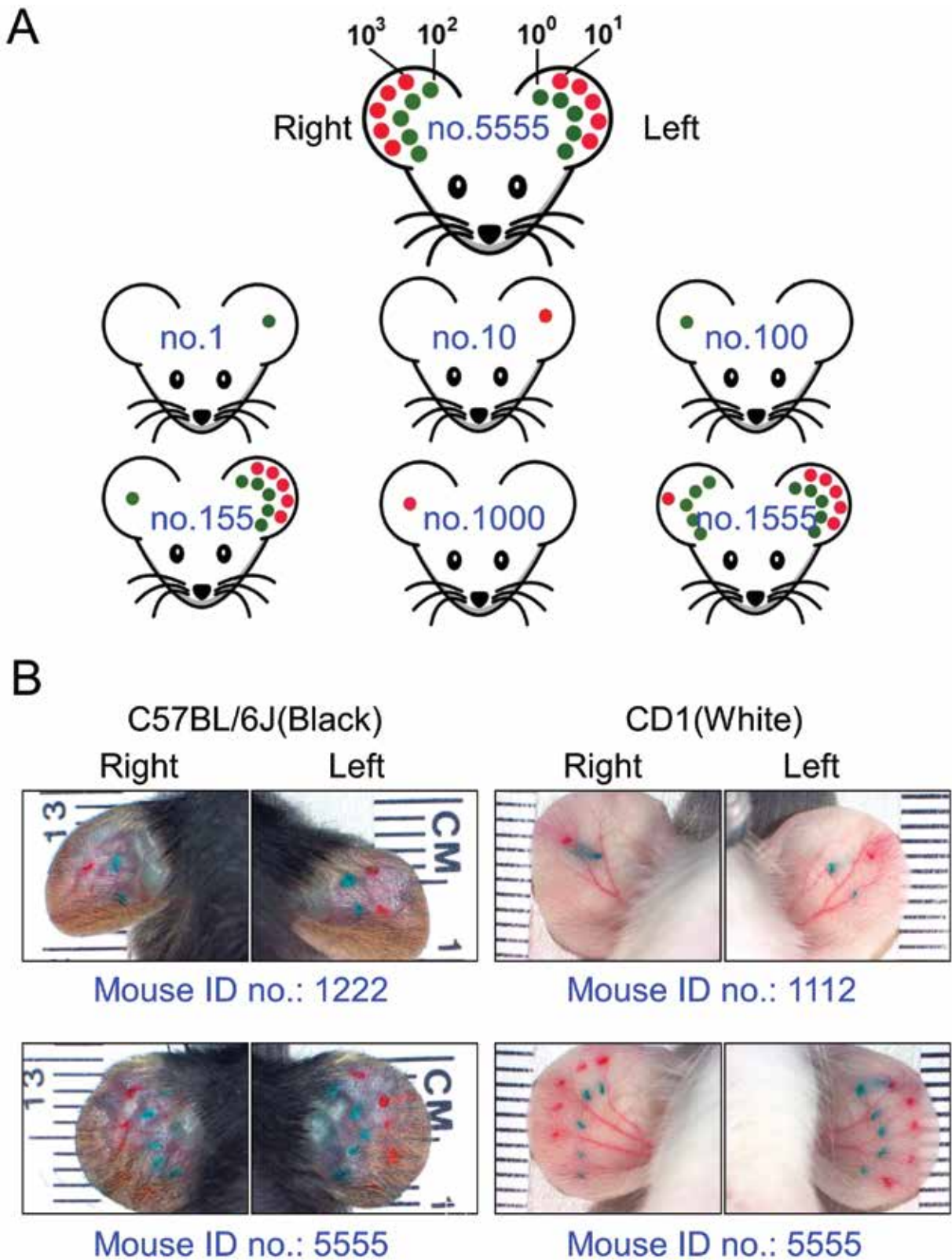
label all other white 11 pups, with similar success in visibility (data not shown).

**Toe tattooing of front feet only to identify black mouse pups.** We used a modification of the classic 4-feet toe-tattooing method





**Figure 4.** An ear-tattooing method in which mouse number is combined with cage number for labeling unlimited adult mice (black or white). (A) A suggested numbering system for ear tattooing (green dots) to label adult mice on the right ear with numbers ranging from 1 to 5 (mouse number); if a mouse is transferred to another cage, the revised marks (transfer numbers 1–5) can be made on the left ear. The panels on the right side are the images of original and transfer-associated revised mouse numbers for white (top) and black (bottom) adult mice. (B) The middle and top rows of this diagram model the transfer of a mouse (no. 4) from cage 1 to cage 2 (where the ‘incoming mouse’ now carries the revised no. 4-1) when cage 2 had no mouse 4 originally. The middle and bottom panels illustrate the transfer of mouse 3 from cage 1 to cage 2 (revised no. 3-1) when cage 2 already had a mouse with the identification code of 3; in this case, the 3 green dots on the right ear of the incoming animal indicate the mouse number and the single green dot on the left ear indicates the cage (or transfer) number. When the cage number is combined with the mouse number, a unique identifier can be generated for each mouse.



**Figure 5.** An ear-tattooing method independent of cage number for labeling adult mice. (A) A suggested ear-tattooing method using a 2-color quinary system to label a maximum of 1295 mice. Possible identification numbers range from 1 to 5555. Representative numbers (1, 10, 100, 155, 1000, and 1555) are displayed. (B) Images of 2-color quinary labeling on black (left, nos. 1222 and 5555) and white (right, nos. 1112 and 5555) mice. This system is sufficient to uniquely label 1295 mice. When more than 1295 mice need to be labeled, cage numbers might be added to increase the number of identification codes possible.

as an alternative approach to identify black mouse pups. Our modified system, which involves the front feet only, can be used for mouse pups of other colors as well. The main advantage of

our modified method is that it keeps the rear toes and limbs intact for use in various experiments or research projects, such as the hindlimb ischemia model. Our numbering system for the

2-feet toe-tattooing approach uniquely identifies a maximum of 14 mouse pups in a single harem-breeding cage (Figure 3 B, top). This method can be applied to adult mice, as necessary (data not shown). In this coding system (Figure 3 B), mice 1 through 8 were labeled by using single marks (1 per digit), with numbers 9 through 14 designated by tattooing 2 adjacent digits. However, this modified toe-tattooing method has its limitations regarding labeling mice when 2 mice with the same marks (each from different cages) have to be transferred into the same cage (for example, for breeding purposes). On weaning, a mouse tattoo number might be combined with the cage number to generate a unique combination for identifying individual mice; alternatively a different method might be used (see following and Figure 4).

**Single-color ear tattooing to identify adult mice.** All of the various classic methods used to identify mice are associated with disadvantages (Figure 1), including misreading and potential side effects on physiologic homeostasis.<sup>10</sup> Here, we propose a novel ear-tattooing approach to minimize these problems. In this approach, a single-color ear tattoo was used to label a maximum of 5 adult mice per cage (the typical maximum). In the associated numbering system (Figure 4 A), 1 to 5 green dots were made on the right ear to indicate mice 1 through 5, whereas 1 to 5 green dots were made on the left ear to indicate the mouse that was transferred. In other words, when a mouse has to be transferred from one cage to another for breeding or other experimental purposes, the left ear is used to indicate the number of times that a mouse has been transferred (maximum of 5 transfers [5 green dots] can be done according to the methods described here). For example, in transferring a mouse transferred from one cage to another (Figure 4 B), 2 possibilities exist. In the first, a mouse (e.g., in Cage 1, Figure 4 B) needs to be transferred to a cage (e.g., Cage 2, Figure 4 B) in which no mouse has the same number as the 'incoming' mouse (Figure 4 B, top and middle panels); here the transferred mouse was tattooed with 1 dot on its left ear to indicate the mouse was a transferred mouse. In the second possible situation, a mouse (e.g., in Cage 1, Figure 4 B) needs to be transferred into a cage (for example, Cage 2, Figure 4 B) that contains a mouse with the same number as the incoming mouse (Figure 4 B, middle and bottom panels); here, the transferred mouse received a green dot on its left ear to indicate the first transfer (Figure 4 B). The single-color ear-tattooing approach can thus be used to uniquely label unlimited numbers of mice when the mouse number is combined with a unique cage number. Because the green animal paste generated clear tattoo marks on both white and black adult mice, we believe that this identification method can be applied to other strains of adult mice, including those with other hair colors and nude mice.

**Using 2-color ear-tattooing to identify adult mice.** The single-color ear-tattooing method required unique cage numbers and additional tattoo sessions for labelling transferred mice. To overcome these potential limitations, we modified this ear-tattooing approach to using 2 colors, in which green and red marks were combined to assign individual adult mice with unique identification codes. As a result, a maximum of 1295 adult mice can be labeled by using unique identification codes that ignore cage numbers. In this system (Figure 5 A), 1 to 5 red dots on the right ear indicated the number in the 1000's place; green dots in the right ear indicated the number in the 100's place; red dots on the left ear represented the number in the 10's place; and green dots on the left ear gave the number in the 1's place. To convert the color-dot combinations into digits, the tattoo dots were read from the mouse's right ear to its left ear, as you face the mouse from the front (Figure 5 A). This coding method can be used to a unique mouse identifier between 1 and 5555; the total number of mice

that can be so identified is only 1295 due to the nonconsecutive numbers of this quinary system. Figure 5 B shows 2 examples of this method used on black and white adult mice.

## Discussion

The present study demonstrated that, by using various combinations of location and ink color, ear, tail, and toe tattooing can be used to uniquely label mice of all ages, from neonatal to adult, regardless of hair or skin color. In particular regarding neonatal mice, 2-color (green and red) tail tattooing is suitable for identifying a maximum of 20 white mouse pups in a single cage; the 2-feet toe-tattooing method can be used for identifying black mouse pups until suitable colors of animal pastes are available commercially. Although the tail-tattooing method can be applied to adult mice, ear-tattooing (single- or 2-color) approaches might be better choices, because these methods can be used to easily and permanently label a large or unlimited number of adult mice without or with using the cage number. In addition, both the single-color and 2-color methods likely can be applied to other strains of adult mice, regardless of hair color.

Animal identification is a common and essential procedure for experimental subjects in research laboratories and animal facilities. About a dozen identification methods (see references 5, 8, and 12 and Figure 1), including ear tagging, ear punching, ear notching, toe clipping, and toe tattooing, have been widely used to label individual experimental subjects from large animals (for example, swine, sheep, and dogs) to small (for example, rabbits, cats, rats, and mice). For lab animals equal to rats in size or larger, the commonly used methods (including some listed in Figure 1) are relatively easy to perform. However, an easy, reliable, noninvasive, safe method is still not available for uniquely labeling mice, the most commonly used lab animal. Ear tagging, ear punching, and toe clipping traditionally have been the most common methods used on mice in the laboratory. These methods are easy to perform, create permanent labels, and provide tissues for genotyping studies. However, several drawbacks of make these methods less attractive: ear tagging may result in short-term negative effects on animal wellbeing;<sup>11</sup> local infection, inflammation, and neoplastic lesions can occur;<sup>3</sup> clipped toes or punched ears can regrow and ear-tags can be lost, resulting in misidentification; and some identification procedures (especially toe clipping) are considered to be inhumane and ethically unjustifiable. Consequently at some research institutes (for example, the NIH's National Human Genome Research Institute), toe clipping is usually not acceptable as the first choice for mouse identification without strong scientific justification.

To overcome these issues, we have proposed several novel tattooing approaches to permanently identify mice of all ages regardless of their skin or hair color. These methods took advantage of microtattooing using a 27- to 30-gauge needle to minimize tissue damage and to reduce potential side effects on physiologic and behavioral parameters, including cannibalism. We determined that a combination of green and red pastes achieved the best marks on the tails of white mouse pups, whereas toe tattooing was used as an alternative approach for labeling black mouse pups until suitable colors of animal tattoo pastes are available commercially. However, for adult mice, our single- or 2-color ear-tattooing method can be used. The maximal number of mice that can be labeled with these methods depends on whether a unique cage number is incorporated into the identification code or not (unlimited or 1295, respectively). Figure 6, provides a flow chart to facilitate the selection of an appropriate method for mouse labeling. The current study represents the first time, to our knowledge, that single- or 2-color ear tattooing was used to label laboratory mice, although





Figure 6. A flow chart to facilitate the selection of an identification method for neonatal or adult mice.

tattoo identification methods (for example, toe tattooing of mice) have been widely used in lab animals.<sup>1</sup> One study<sup>6</sup> reported the application of a single-color (green) ear microtattoo on white rats, but the study focused on the short-term effects of the identification method on the blood pressure of rats, and no 2-color applications or any numbering systems were described.

Our proposed ear-tattooing method and numbering system are easy to apply, needing only a few tools and minimal practice. The ear-, tail-, and toe-tattooing methods we describe likely cause less pain and discomfort, tissue damage, and stress to mice than do toe clipping or ear notching. In addition, the red and green tattooed marks are clear and permanent on white

and black adult mice and white neonatal mice. In the future, we will reexamine tattooing the tails of neonatal black mice when suitable colors of animal tattooing pastes are available commercially. We recommend using a combined method of tail, toe, and ear tattooing to uniquely label individual mice, from neonatal to adult, in combination with a nonrepeated cage number. Tail and toe tattoos should be considered as temporary labels for neonatal mice before their weaning at PND 21 if these mice are used in the projects, in which the tail (for example, injection or genotyping) or toes (for example, toe or limb regeneration) have to be kept intact without any damage. However, for the sake of consistency, the same identification code used for the neonatal tail tattoo should be reapplied as an ear tattoo after weaning. The ear-tattooing approach can be used to add permanent labels in all types of adult mice, in which a single-color ear-tattoo system (using a unique cage number) seems to be the best choice of identification method (Figure 6).

The modified methods we present here had several weaknesses (Figure 1). For example, although apparently ideal for adult mice, single-color ear tattooing required the use of a unique cage number, and intercage transfers were limited (maximum of 5 transfers). In contrast, 2-color ear tattooing provides a unique label that is independent of cage number, but the total number of mice that can be labeled uniquely is limited to a maximum of 1295. In addition, a combined 2-color tail-tattooing method works well for white mouse pups (using red and green pastes) but has yet to be adapted effectively for black pups. Further red–green tattooing may be suboptimal for researchers who have red–green blindness. Moreover, we observed that mice occasionally licked off excess tattoo paste from the ear, tail, or toes of another mouse; this drawback can be minimized by limiting the amount of paste on the needle during tattooing. Finally, the cannibalism that is observed during routine mice care is a rare event and seems unrelated to the identification procedures used in the present study, given that cannibalism also occurred in a breeding cage that had not experienced any identification procedures.

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