
1. PURPOSE

This Standard Operating Procedure (SOP) describes acceptable methods for collection of tissue samples to be used for genotyping in mice.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. MATERIALS

- 3.1. Antiseptic for skin (e.g., 70% alcohol, chlorhexidine, povidone-iodine)
- 3.2. Sharp surgical scissors or sterile, disposable scalpel blades
- 3.3. Ear punch
- 3.4. Gauze
- 3.5. 70% alcohol (for sanitizing instruments)
- 3.6. Sterile cotton-tipped swabs
- 3.7. Collection tubes, properly identified
- 3.8. Chemical cautery agent (tissue glue, Kwik Stop® topical styptic powder or silver nitrate)
- 3.9. Glass bead sterilizer
- 3.10. Anesthetics
- 3.11. Analgesics

4. PROCEDURES

- 4.1. Fecal pellet:
 - 4.1.1. Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding.
 - 4.1.2. Identify animal as per Rodent Identification SOP.
 - 4.1.3. Place fecal pellet in an identified collection tube.
 - 4.1.4. Note that this method may or may not provide sufficient DNA.
- 4.2. Skin swabbing:
 - 4.2.1. Restrain the animal.
 - 4.2.2. Using a cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth. Perform a minimum of 3 strokes of 3cm in length each.
 - 4.2.3. Insert cotton bud into collection tube and snip off excess shaft.
 - 4.2.4. Identify animal as per Rodent Identification SOP.
 - 4.2.5. Note that this method may or may not provide sufficient DNA.
- 4.3. Buccal epithelial cell:
 - 4.3.1. Firmly restrain the animal by the scruff to maintain its mouth open.
 - 4.3.2. Using a cotton-tipped swab with a <2mm bud, vigorously rub the inner cheeks while rotating the swab, avoiding the tongue.
 - 4.3.3. Insert cotton bud into collection tube and snip off excess shaft.
 - 4.3.4. Identify animal as per Rodent Identification SOP.
 - 4.3.5. Note that this method may or may not provide sufficient DNA.

- 4.4. Ear punching:
 - 4.4.1. Do not use this method in rodents under 2 weeks of age.
 - 4.4.2. Ensure the ear punch apparatus is not dull.
 - 4.4.3. Disinfect the ear punch with 70% alcohol and wipe dry.
 - 4.4.4. Restrain the animal securely by the scruff.
 - 4.4.5. Using the ear punch, punch holes and/or notches in the ears, following an identification chart. See sample in annex.
 - 4.4.6. Use the excised tissue as a sample for genotyping. Place in well-identified collection tube
 - 4.4.7. Disinfect ear punch between mice.
- 4.5. Tail biopsy:
 - 4.5.1. Tail snipping should be performed on mice between 14 and 21 days of age (ideally between 14 and 17 days).
 - 4.5.2. Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
 - 4.5.3. Identify animal as per Rodent Identification SOP.
 - 4.5.4. Tail snipping procedure for mice less than 21 days of age:
 - 4.5.4.1. General anesthesia is recommended but not required
 - 4.5.4.2. Gently, but securely, restrain mouse (manual or mechanical).
 - 4.5.4.3. Swab the tail with antiseptic (e.g. alcohol).
 - 4.5.4.4. Snip 2-3mm off the tip of the tail with sharp, sanitized scissors or disposable scalpel.
 - 4.5.4.5. Remove biologic material and sanitize the scissors after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you are snipping several mice tails.
 - 4.5.4.6. Place tissue sample into an identified collection tube.
 - 4.5.4.7. Check for bleeding before returning mouse to its cage. If bleeding occurs, do one of the following:
 - 4.5.4.7.1. Apply a drop of tissue glue to tip of tail.
 - 4.5.4.7.2. Apply a chemical cauterly agent (e.g. Kwik Stop® powder or silver nitrate stick).
 - 4.5.4.7.3. Electric or heat cauterize the cut end of the tail
 - 4.5.5. Tail snipping procedure for mice over 21 days of age:
 - 4.5.5.1. Requires general anesthesia and analgesia.
 - 4.5.5.2. Brief general anesthesia is provided with isoflurane, by placing the mouse in an induction chamber to achieve unconsciousness. Refer to Mouse Anesthesia and Rodent Analgesia SOPs.
 - 4.5.5.3. Perform the tail snipping as defined in sections 4.5.4.3 to 4.5.4.7 of this SOP.
- 4.6. Whole blood:
 - 4.6.1. Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.
 - 4.6.2. Note that this method may or may not provide sufficient DNA.
- 4.7. Distal phalanx biopsy:
 - 4.7.1. This method is acceptable only under the following conditions:
 - 4.7.1.1. The genotype needs to be known before weaning. This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method
 - 4.7.1.2. Mice must be approximately 7 days old at the time of the biopsy.
 - 4.7.1.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.
 - 4.7.1.4. Only the most distal phalanx can be removed
 - 4.7.1.5. Sharp iris scissors must be used.
 - 4.7.1.6. No further biopsy can be performed.

5. REFERENCES

- 5.1. 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*. Nov;47(6):10-8 (2008).
- 5.2. CompMed listserv; American Association for Laboratory Animal Science (AALAS): Meldgaard M, Bollen PJ, Finsen B. "Non-invasive method for sampling and extraction of mouse DNA for PCR". *Laboratory Animals* 38, 413–417(2004).
- 5.3. Mitrečić D, Mavrić S, Branica BV, Gajović S. "Mice genotyping using buccal swab samples: an improved method". *Biochem Genet* 46:105–112 (2008).
- 5.4. Cinelli, P., Rettich, A, Seifert, B, Bürki, K. and M.Arras. "Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice". *Laboratory Animals* 41, 174–184 (2007).
- 5.5. Picazo MG, García-Olmo DC. "DNA from tissues of young mice is optimal for genotyping". *Electronic Journal of Biotechnology*. Available online 8 January 2015, <http://www.sciencedirect.com/science/article/pii/S071734581400147X>
- 5.6. Bonaparte D *et al*. "FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents." *Laboratory Animals* 47(3) (2013).
- 5.7. Castelhana-Carlos MJ, Sousa N, Ohl F and Baumans V. "Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation" *Laboratory Animals* 44: 88–103 (2010)
- 5.8. Okada M, Miller TC, Roediger J, Shi YB, Schech JM. "An Efficient, Simple, and Noninvasive Procedure for Genotyping Aquatic and Nonaquatic Laboratory Animals." *J Am Assoc Lab Anim Sci* 47, p10.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2016.03.17	4.5 (NO TEXT)	<p>4.5. Distal phalanx biopsy:</p> <p>4.5.1. This method is acceptable only under the following conditions:</p> <p>4.5.1.1. The genotype needs to be known before weaning. This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method</p> <p>4.5.1.2. Mice must be approximately 7 days old at the time of the biopsy.</p> <p>4.5.1.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.</p> <p>4.5.1.4. Only the most distal phalanx can be removed</p> <p>4.5.1.5. Sharp iris scissors must be used.</p> <p>4.5.1.6. No further biopsy can be performed.</p>
2017.12.12	3.6 Aluminum cotton-tipped swab (<2mm bud)	3.6 Aluminum Sterile cotton-tipped swab (<2mm bud)
2017.12.12	4.2 Buccal epithelial cell	4.2 4.3 Buccal epithelial cell
2017.12.12	4.3.2. Using a cotton-tipped swab, vigorously scrape the inner cheeks, avoiding the tongue.	4.3.2. Using a cotton-tipped swab with a <2mm bud, vigorously scrape rub the inner cheeks while rotating the swab, avoiding the tongue.
2017.12.12	4.2 (NO TEXT)	<p>4.2 Skin swabbing:</p> <p>4.2.1 Restrain the animal.</p> <p>4.2.2 Using a cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth. Perform a minimum of 3 strokes of 3cm in length each.</p> <p>4.2.3 Insert cotton bud into collection tube and snip off excess shaft.</p> <p>4.2.4 Identify animal as per Rodent Identification SOP.</p> <p>4.2.5 Note that this method may or may not provide sufficient DNA.</p>
2017.12.12	4.5.4.7. Check for bleeding	4.5.4.7. Check for bleeding before returning mouse to its cage.
2017.12.12	4.4.4.8. Return the mouse to its cage.	4.4.4.8. Return the mouse to its cage.
2017.12.12	4.6 (NO TEXT)	<p>4.6. Whole blood:</p> <p>4.6.1. Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.</p>
2017.12.12	5.8 (NO TEXT)	5.8 Okada M, Miller TC, Roediger J, Shi YB, Schech JM. "An Efficient, Simple, and Noninvasive Procedure for Genotyping Aquatic and Nonaquatic Laboratory Animals." <i>J Am Assoc Lab Anim Sci</i> , 2017 Jul 3.

Sample Ear Notching Code

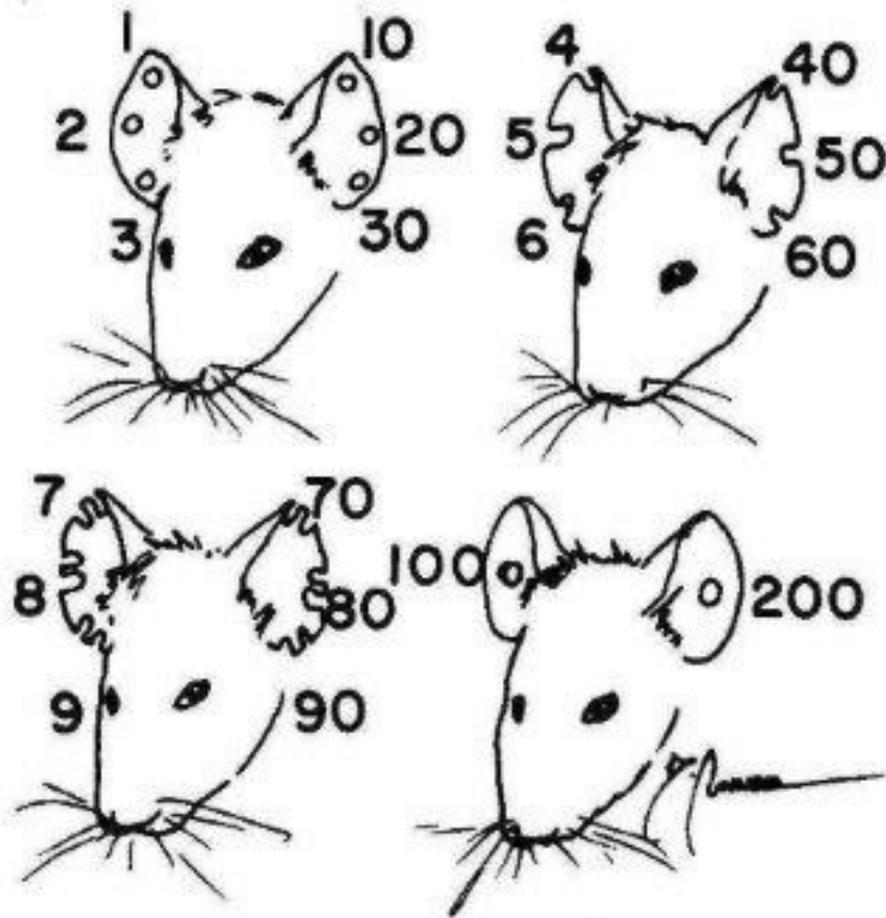


Fig. 1. Ear notch-punch code for identification of rodents. These number codes are used in various combinations to produce the desired number.