

1. PURPOSE

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

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. Aluminum cotton-tipped swab (<2mm bud)
- 3.7. Collection tubes
- 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.
- 4.2. Buccal epithelial cell:
 - 4.2.1. Firmly restrain the animal by the scruff to maintain its mouth open.
 - 4.2.2. Using a cotton-tipped swab, vigorously scrape the inner cheeks, avoiding the tongue.
 - 4.2.3. Insert cotton bud into collection tube and snip off excess shaft.
 - 4.2.4. Identify animal as per Rodent Identification SOP.
- 4.3. Ear punching:
 - 4.3.1. Do not use this method in rodents under 2 weeks of age.
 - 4.3.2. Restrain the animal securely.
 - 4.3.3. Using the ear punch, punch holes and/or notches in the ears, following an identification chart. See sample chart in annex.
 - 4.3.4. Use the excised tissue as a sample for genotyping.

4.4. Tail snipping:

- 4.4.1. Tail snipping should be performed on rats between 14 and 21 days of age (ideally between 14 and 17 days).
- 4.4.2. Remove 2-3mm of tail tip. Tail biopsy can only be performed twice over the life time of the animal and cannot exceed 5mm total.
- 4.4.3. Identify animal as per Rodent Identification SOP.
- 4.4.4. Tail snipping procedure for rats less than 21 days of age:
 - 4.4.4.1. General anesthesia is recommended but not required.
 - 4.4.4.2. Gently, but securely, restrain the rat (manual or mechanical).
 - 4.4.4.3. Swab the tail with antiseptic (e.g. alcohol).
 - 4.4.4.4. Snip tail with sharp, sanitized scissors or disposable scalpel.
 - 4.4.4.5. 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 tails.
 - 4.4.4.6. Place tissue sample into a collection tube.
 - 4.4.4.7. Check for bleeding. If bleeding occurs, do one of the following:
 - 4.4.4.7.1. Apply a drop of tissue glue to tip of tail.
 - 4.4.4.7.2. Apply a chemical cautery agent (e.g. Kwik Stop® powder or silver nitrate stick).
 - 4.4.4.7.3. Electric or heat cauterize the cut end of the tail
 - 4.4.4.8. Return the rat to its cage.
- 4.4.5. Tail snipping procedure for rats over 21 days of age:
 - 4.4.5.1. Requires general anesthesia and analgesia.
 - 4.4.5.2. Brief general anesthesia is provided with isoflurane, by placing the rat in an induction chamber to achieve unconsciousness. Refer to Rat Anesthesia and Rodent Analgesia SOPs.
 - 4.4.5.3. Perform the tail snipping as defined in sections 4.4.4.3 to 4.4.4.8 of this SOP.

4.5. Blood sampling:

- 4.5.1. Collect blood from the saphenous vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.
- 4.5.2. Identify animal as per Rodent Identification SOP.

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. Guidelines on Genetically-Engineered Animals, 2nd Draft; Canadian Council on Animal Care (CCAC): http://ccac.ca/gea_downloads/GEA_Guidelines_Second_Draft_18Aug08.pdf
- 5.3. CompMed listserv; American Association for Laboratory Animal Science (AALAS): http://www.aalas.org/online_resources/listserves.asp#compmed
- 5.4. Meldgaard M, Bollen PJ, Finsen B. "Non-invasive method for sampling and extraction of mouse DNA for PCR". *Laboratory Animals* 38, 413–417(2004).
- 5.5. Mitrečić D, Mavrić S, Branica BV, Gajović S. "Mice genotyping using buccal swab samples: an improved method". *Biochem Genet* 46:105–112 (2008).
- 5.6. 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).

Sample Ear Notching Code

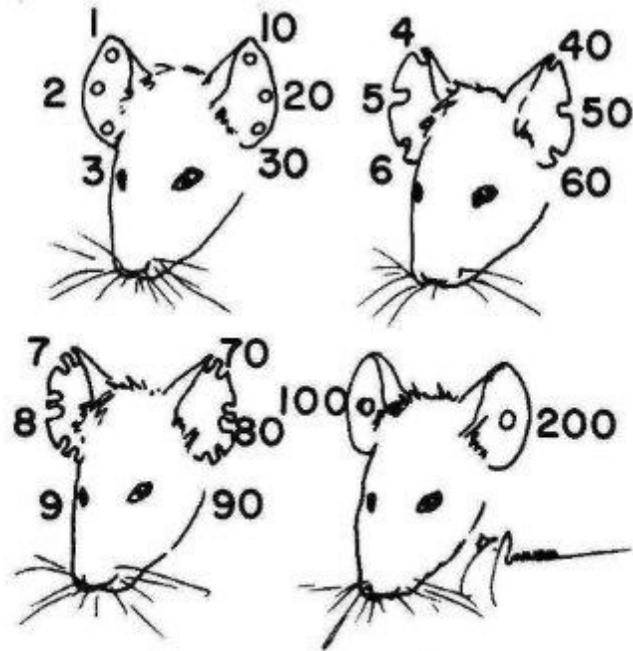


Fig. 1. Ear notch-punch code for identification of rodents. These number codes are used in various combinations to produce the desired number.