McGill

STANDARD OPERATING PROCEDURE #413

# TISSUE COLLECTION FOR GENOTYPING - RATS

## 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 and their research staff, veterinary care staff.

## 3. MATERIALS

3.1. Sharp surgical scissors or sterile, disposable scalpel blades

3.2. Ear punch

3.3. Gauze

3.4. 70% alcohol (for sanitizing instruments)

3.5. Aluminum cotton-tipped swab (<2mm bud)

3.6. Collection tubes

3.7. Tissue glue (Vetbond®)

3.8. Glass bead sterilizer

3.9. Anesthetics

3.10. Analgesics

## 4. CONSIDERATIONS

4.1. The least invasive tissue collection method available, i.e., the method causing the least discomfort to the animals, should be selected. Methods are listed in in this SOP according to the potential pain and distress for the animals, from the least invasive to the most invasive.

4.2. Since animals must be individually identified at the time of tissue collection for genotyping, a method that provides a DNA sample at the same time as it identifies the animal, e.g., ear punching, should be prioritized. This minimizes the number of procedures carried out on the animals, and hence minimizes pain and distress.

4.3. The selection of the tissue collection method should take into consideration the age of the animals at the time of tissue collection. In general, biopsies from young rats result in larger amounts of pure DNA than those from adult rats.

## 5. PROCEDURES

5.1. Fecal pellet:

5.1.1. Stools contain sloughed intestinal epithelial cells which provide a reliable source of DNA for genotyping.

5.1.2. Fresh fecal pellets must be used; genotyping should be performed within 24 hours of collection. More than one fecal pellet per animal is usually required.

5.1.3. Collect fecal pellet from an individual animal using brief manual restraint or place individual animals in a clean cage without bedding.

5.1.4. This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sample collection process.

5.1.5. Identify animal as per Rodent Identification SOP.

5.1.6. Place fecal pellet in an identified collection tube.

5.2. Skin swabbing:

5.2.1. The DNA isolated from skin swabbing can be minimal and may be difficult to measure by conventional methods.

5.2.2. This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sample collection process.

5.2.3. Restrain the animal.

5.2.4. Using a sterile 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.

5.2.5. Insert cotton bud into collection tube and snip off excess shaft.

5.2.6. Identify animal as per Rodent Identification SOP.

5.3. Buccal epithelial cell:

5.3.1. This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sample collection process.

5.3.2. Firmly restrain the animal by the scruff to maintain its mouth open.

5.3.3. Using a cotton-tipped swab with a <2mm bud, vigorously scrape the inner cheeks while rotating the swab, avoiding the tongue.

5.3.4. Insert cotton bud into collection tube and snip off excess shaft.

5.3.5. Identify animal as per Rodent Identification SOP.

5.4. Ear punching/notching:

5.4.1. Do not use this method in rodents under 2 weeks of age as the pinna is not yet fully developed.

5.4.2. The use of a 2 mm ear punch is recommended as this will yield sufficient DNA and will ensure the identification is not lost after healing.

5.4.3. Ensure the ear punch apparatus is not dull.

5.4.4. Disinfect the ear punch or scissors with 70% alcohol and wipe dry.

5.4.5. Restrain the animal securely by the scruff.

5.4.6. Using the ear punch, punch holes and/or notches in the ears, following an identification chart. See sample in annex.

5.4.7. Use the excised tissue as a sample for genotyping. Place in well-identified collection tube

5.4.8. Disinfect ear punch or scissors between animals.

5.5. Whole blood:

5.5.1. Collect as per SOP 403-Guidelines Blood Collection Volumes and Frequency.

5.6. Tail biopsy:

5.6.1. The tail biopsy is considered an invasive procedure since nerves, bones/cartilage, connective tissue, ligaments, and skin are being severed.

5.6.2. Tail biopsy is ideally performed on rodents before 17 days of age to avoid transection of distal mature vertebrae. When collected before 17 days of age, the tail biopsy sample will be less ossified and will provide better quality DNA and higher DNA yield.

5.6.3. Minimize the amount of tissue removed; 2 mm of distal tail has been identified as sufficient tissue to perform multiple PCR reactions. The tail biopsy sample cannot exceed 5mm.

5.6.4. Tail biopsy should only be performed once over the lifetime of the animal.

5.6.5. Identify animal as per Rodent Identification SOP.

5.6.6. Tail snipping procedure for rats less than 21 days of age:

5.6.6.1. General anesthesia is recommended but not required.

5.6.6.2. Gently, but securely, restrain the rat.

5.6.6.3. Snip 2-3mm off the tip of the tail with sharp, sanitized scissors or disposable scalpel.

5.6.6.4. Remove biologic material and sanitize the scissors or scalpel after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you are snipping several tails.

5.6.6.5. Place tissue sample into an identified collection tube.

5.6.6.6. Check for bleeding before returning animal to its cage. If bleeding occurs, apply a drop of tissue glue to tip of tail.

5.6.7. Tail snipping procedure for rats over 21 days of age:

5.6.7.1. Tail biopsy is not the method of choice for tissue collection in rats aged over 21 days of age. A less-invasive alternative method for collecting the tissue sample should be used.

5.6.7.2. When tail biopsy samples are to be collected in rats over 21 days of age, the procedure is to be described in the approved Animal Use Protocol and scientific justification for selecting this method must be provided.

5.6.7.3. General anesthesia and analgesia are required. Refer to Rat Anesthesia and Rodent Analgesia SOPs.

5.6.7.4. Perform the tail snipping as defined in sections 5.6.6.2 to 5.6.6.6 of this SOP.

5.7. Distal phalanx biopsy:

5.7.1. This method must be described in the approved Animal Use Protocol and scientific justification for selecting this method must be provided. Distal phalanx biopsy is acceptable only under the following conditions:

5.7.1.1. The genotype needs to be known before 14 days of age. This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method

5.7.1.2. Other methods, such a tail biopsy for tissue collection and microtattoo of toes or tails for identification, have been shown not to be successful.

5.7.1.3. Rats must be under 7 days old at the time of the biopsy.

5.7.1.4. General analgesia must be provided. Refer to Rodent Analgesia SOP.

5.7.1.5. Only one toe is to be clipped.

5.7.1.6. Only the most distal phalanx can be removed

5.7.1.7. No further biopsy can be performed.

5.7.2. Distal phalanx biopsy procedure:

5.7.2.1. Sharp iris scissors must be used.

5.7.2.2. Avoid clipping digits/toes on fore paws if possible.

5.7.2.3. Do not clip the first digit/toe, i.e., thumb, on either fore paw.

5.7.2.4. Only remove the third phalanx, i.e., the last and most distal bone of a digit; amputate at the joint between the second and third phalanges.

5.7.2.5. Remove biologic material and sanitize the scissors (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) between animals.

5.7.2.6. Place tissue sample into an identified collection tube.

5.7.2.7. Check for bleeding before returning animal to its cage. If bleeding occurs, apply a drop of tissue glue to tip of toe.

## 6. REFERENCES


6.1. 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).

6.2. Guidelines on Genetically-Engineered Animals, 2nd Draft; Canadian Council on Animal Care (CCAC): http://ccac.ca/gea_downloads/GEA_Guidelines_Second_Draft_18Aug08.pdf

6.3. Meldgaard M, Bollen PJ, Finsen B. “Non-invasive method for sampling and extraction of mouse DNA for PCR”. *Laboratory Animals* 38, 413–417(2004).

6.4. Mitrecić D, Mavrić S, Branica BV, Gajović S. “Mice genotyping using buccal swab samples: an improved method”. *Biochem Genet* 46:105–112 (2008).

6.5. 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).

6.6. 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

6.7. Bonaparte D *et al. “*FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents*.” Laboratory Animals* 47(3) (2013). https://doi.org/10.1177/0023677212473918

6.8. Castelhano-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)

6.9. 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.

6.10. Canadian Council on Animal Care. CCAC Guidelines: Mice. (2019).

6.11. Koh JY, Iwabuchi S, Huang Z, Harata NC. Rapid genotyping of animals followed by establishing primary cultures of brain neurons. J Vis Exp. 2015;(95):51879. Published 2015 Jan 29. doi:10.3791/51879

6.12. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. Lab Anim. 2013 Jan;47(1):2-11. doi: 10.1177/002367712473290. PMID: 23467487.

6.13. Chen M, Kan L, Ledford BT, He JQ. Tattooing Various Combinations of Ears, Tail, and Toes to Identify Mice Reliably and Permanently. J Am Assoc Lab Anim Sci. 2016 Mar;55(2):189-98. PMID: 27025811; PMCID: PMC4783638.

## SOP REVISION HISTORY

| DATE | REVISIONS |
|---|---|
| 2019.11.24 | 4.3. Ear punching**/notching:**<br>4.3.1. Do not use this method in rodents under 2 weeks of age.<br>**4.3.2. Ear punches or notches should be no larger than 2mm.**<br>**4.3.3. Ensure the ear punch apparatus or scissors are sharp.**<br>**4.3.4. Disinfect the ear punch or scissors with 70% alcohol and wipe dry.**<br>4.3.5. Restrain the animal securely.<br>4.3.6. Using the ear punch, punch holes and/or notches in the ears, following an identification chart. **Alternatively, use scissors to make small notches in the ears.** See sample in annex.<br>4.3.7. Use the excised tissue as a sample for genotyping. **Place in well-identified collection tube.**<br>**4.3.8. Disinfect ear punch or scissors between animals.** |
| 2019.11.24 | **4.6. Distal phalanx biopsy:**<br>**4.6.1. This method is acceptable only under the following conditions:**<br>**4.6.1.1. 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.**<br>**4.6.1.2. Rats must be approximately 7 days old at the time of the biopsy.**<br>**4.6.1.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.**<br>**4.6.1.4. Only the most distal phalanx can be removed**<br>**4.6.1.5. Sharp iris scissors must be used.**<br>**4.6.1.6. No further biopsy can be performed.** |
| 2022.07.12 | ~~3.1. Antiseptic for skin (e.g., 70% alcohol, chlorhexidine, povidone-iodine)~~ |
| 2022.07.12 | ~~3.7. Chemical cautery agent (~~Tissue glue **(Vetbond®)**~~, Kwik Stop® topical styptic powder or silver nitrate)~~ |
| 2022.07.12 | **4. CONSIDERATIONS**<br>**4.1. The least invasive tissue collection method available, i.e., the method causing the least discomfort to the animals, should be selected. Methods are listed in in this SOP according to the potential pain and distress for the animals, from the least invasive to the most invasive.**<br>**4.2. Since animals must be individually identified at the time of tissue collection for genotyping, a method that provides a DNA sample at the same time as it identifies the animal, e.g., ear punching, should be prioritized. This minimizes the number of procedures carried out on the animals, and hence minimizes pain and distress.**<br>**4.3. The selection of the tissue collection method should take into consideration the age of the animals at the time of tissue collection. In general, biopsies from young rats result in larger amounts of pure DNA than those from adult rats.** |
| 2022.07.12 | **5.1.1. Stools contain sloughed intestinal epithelial cells which provide a reliable source of DNA for genotyping.**<br>**5.1.2. Fresh fecal pellets must be used; genotyping should be performed within 24 hours of collection. More than one fecal pellet per animal is usually required.** |
| 2022.07.12 | 5.1.3. Collect fecal pellet from an individual animal using brief manual restraint or ~~by placing it~~ **place individual animals** in a clean cage without bedding.<br>**5.1.4. This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sample collection process.** |

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|---|---|
| 2022.07.12 | **5.2. Skin swabbing:**<br>**5.2.1. The DNA isolated from skin swabbing can be minimal and may be difficult to measure by conventional methods.**<br>**5.2.2. This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sampling process.**<br>**5.2.3. Restrain the animal.**<br>**5.2.4. Using a sterile 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.**<br>**5.2.5. Insert cotton bud into collection tube and snip off excess shaft.**<br>**5.2.6. Identify animal as per Rodent Identification SOP.** |
| 2022.07.12 | 5.3.1. **This method can be prone to cross-contamination and may not be suitable for all studies. Care must be taken to prevent cross-contamination during the sample collection process.**<br>5.3.3. Using a cotton-tipped swab **with a <2mm bud**, vigorously scrape the inner cheeks **while rotating the swab**, avoiding the tongue. |
| 2022.07.12 | 5.4.1. Do not use this method in rodents under 2 weeks of age **as the pinna is not yet fully developed**.<br>5.4.2. ~~Ear punches or notches should be no larger than 2mm.~~ **The use of a 2 mm ear punch is recommended as this will yield sufficient DNA and will ensure the identification is not lost after healing.**<br>5.4.3. Ensure the ear punch apparatus ~~or scissors are sharp~~ **is not dull**.<br>5.4.4. Disinfect the ear punch ~~or scissors~~ with 70% alcohol and wipe dry.<br>5.4.5. Restrain the animal securely by the scruff.<br>5.4.6. Using the ear punch, punch holes and/or notches in the ears, following an identification chart. ~~Alternatively, use scissors to make small notches in the ears.~~ See sample in annex. |
| 2022.07.12 | 5.5. ~~Blood sampling~~ **Whole blood**:<br>5.5.1. Collect ~~blood from the saphenous vein. Refer to~~**as per SOP 403** Guidelines for Blood Collection Volumes and Frequency ~~SOP~~.<br>~~5.5.2. Identify animal as per Rodent Identification SOP.~~ |
| 2022.07.12 | 5.6. Tail ~~snipping~~ **biopsy**:<br>**5.6.1. The tail biopsy is considered an invasive procedure since nerves, bones/cartilage, connective tissue, ligaments, and skin are being severed.**<br>5.6.2. ~~Tail snipping should be performed on rats between 14 and 21 days of age (ideally between 14 and 17 days).~~ **Tail biopsy is ideally performed on rodents before 17 days of age to avoid transection of distal mature vertebrae. When collected before 17 days of age, the tail biopsy sample will be less ossified and will provide better quality DNA and higher DNA yield.**<br>5.6.3. ~~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.~~ **Minimize the amount of tissue removed; 2 mm of distal tail has been identified as sufficient tissue to perform multiple PCR reactions. The tail biopsy sample cannot exceed 5mm.**<br>**5.6.4. Tail biopsy should only be performed once over the lifetime of the animal.** |
| 2022.07.12 | 5.6.6.2. Gently, but securely, restrain the rat ~~(manual or mechanical).~~<br>~~5.6.6.3. Swab the tail with antiseptic (e.g. alcohol).~~<br>5.6.6.4. Snip **2-3mm off the tip of the** tail with sharp, sanitized scissors or disposable scalpel.<br>5.6.6.4. Remove biologic material and sanitize the scissors **or scalpel** after each snipping (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) if you are snipping several tails.<br>5.6.6.5. Place tissue sample into **an identified** collection tube.<br>5.6.6.6. Check for bleeding before returning animal to its cage. If bleeding occurs, **apply a drop of tissue glue to tip of tail.** ~~do one of the following:~~<br>~~5.6.6.7.1. Apply a drop of tissue glue to tip of tail.~~<br>~~5.6.6.7.2. Apply a chemical cautery agent (e.g. Kwik Stop® powder or silver nitrate stick).~~<br>~~5.6.6.7.3. Electric or heat cauterize the cut end of the tail~~<br>~~5.6.6.8. Return the rat to its cage.~~ |
| 2022.07.12 | **5.6.7.1. Tail biopsy is not the method of choice for tissue collection in rats aged over 21 days of age. A less-invasive alternative method for collecting the tissue sample should be used.**<br>**5.6.7.2. When tail biopsy samples are to be collected in rats over 21 days of age, the procedure is to be described in the approved Animal Use Protocol and scientific justification for selecting this method must be provided.**<br>**5.6.7.3.** ~~Requires~~ General anesthesia and analgesia **are required. Refer to Rat Anesthesia and Rodent Analgesia SOPs.**<br>~~5.6.7.4. 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.~~ |
| 2022.07.12 | 5.7.1. This method ~~is acceptable only~~ **must be described in the approved Animal Use Protocol and scientific justification for selecting this method must be provided. Distal phalanx biopsy is acceptable only** under the following conditions:<br>5.7.1.1. The genotype needs to be known before ~~weaning~~ **14 days of age**. **This method replaces the tail biopsy as a sample for genotyping and ear notching as an identification method.**<br>**5.7.1.2. Other methods, such a tail biopsy for tissue collection and microtattoo of toes or tails for identification, have been shown not to be successful.**<br>5.7.1.3. Rats must be ~~approximately~~ **under** 7 days old at the time of the biopsy.<br>**5.7.1.4. General analgesia must be provided. Refer to Rodent Analgesia SOP.**<br>~~5.7.1.5. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.~~ **Only one toe is to be clipped.**<br>**5.7.1.7. Sharp iris scissors must be used.** |
| 2022.07.12 | **5.7.2. Distal phalanx biopsy procedure:**<br>**5.7.2.1. Sharp iris scissors must be used.**<br>**5.7.2.2. Avoid clipping digits/toes on fore paws if possible.**<br>**5.7.2.3. Do not clip the first digit/toe, i.e., thumb, on either fore paw.**<br>**5.7.2.4. Only remove the third phalanx, i.e., the last and most distal bone of a digit; amputate at the joint between the second and third phalanges.**<br>**5.7.2.5. Remove biologic material and sanitize the scissors (wipe with 70% alcohol or dip in glass bead sterilizer for at least 30 seconds) between animals.**<br>**5.7.2.6. Place tissue sample into an identified collection tube.**<br>**5.7.2.7. Check for bleeding before returning animal to its cage. If bleeding occurs, apply a drop of tissue glue to tip of toe.** |
| 2022.07.12 | ~~5.3. CompMed listserv; American Association for Laboratory Animal Science (AALAS): http://www.aalas.org/online_resources/listserves.asp#compmed~~<br>**6.6. 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**<br>**6.7. Bonaparte D et al. "FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents." Laboratory Animals 47(3) (2013). https://doi.org/10.1177/0023677212473918**<br>**6.8. Castelhano-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)**<br>**6.9. 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.**<br>**6.10. Canadian Council on Animal Care. CCAC Guidelines: Mice. (2019).**<br>**6.11. Koh JY, Iwabuchi S, Huang Z, Harata NC. Rapid genotyping of animals followed by establishing primary cultures of brain neurons. J Vis Exp. 2015;(95):51879. Published 2015 Jan 29. doi:10.3791/51879**<br>**6.12. Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. Lab Anim. 2013 Jan;47(1):2-11. doi: 10.1177/002367712473290. PMID: 23467487.**<br>**6.13. Chen M, Kan L, Ledford BT, He JQ. Tattooing Various Combinations of Ears, Tail, and Toes to Identify Mice Reliably and Permanently. J Am Assoc Lab Anim Sci. 2016 Mar;55(2):189-98. PMID: 27025811; PMCID: PMC4783638.** |

# Sample Ear Notching Charts