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 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 as 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


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 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 provides an identification method.

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.

4.4. Collect fecal pellet from an individual animal using brief manual restraint or by placing it in a clean cage without bedding.

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 by placing it in a clean cage without bedding. 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.

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

4.3.6. Using the ear punch, punch holes and/or notches in the ears, following an identification chart.

4.3.8. Disinfect ear punch or scissors between animals.

4.5.1.6. Sharp iris scissors must be used.

4.5.2. Rats must be approximately 7 days old at the time of the biopsy.

4.5.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

4.5.4. 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.

4.5.5. 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 this SOP according to the potential pain and distress for the animals, from the least invasive to the most invasive.

4.5.6.1. 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.

4.5.6.2. Rats must be approximately 7 days old at the time of the biopsy.

4.5.6.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

4.5.6.4. 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.

4.5.6.5. 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 this SOP according to the potential pain and distress for the animals, from the least invasive to the most invasive.

4.5.6.6. Sharp iris scissors must be used.

4.5.6.7. No further biopsy can be performed.

4.6. Distal phalanx biopsy:

4.6.1. This method is acceptable only under the following conditions:

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.

4.6.1.2. Rats must be approximately 7 days old at the time of the biopsy.

4.6.1.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

4.6.1.4. Only the most distal phalanx can be removed.

4.6.1.5. Sharp iris scissors must be used.

4.6.1.6. No further biopsy can be performed.

4.6.1.7. 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.

4.6.1.8. Rats must be approximately 7 days old at the time of the biopsy.

4.6.1.9. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

4.6.1.10. Only the most distal phalanx can be removed.

4.6.1.11. Sharp iris scissors must be used.

4.6.1.12. No further biopsy can be performed.

4.6.2.1. 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.

4.6.2.2. Rats must be approximately 7 days old at the time of the biopsy.

4.6.2.3. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

4.6.2.4. Only the most distal phalanx can be removed.

4.6.2.5. Sharp iris scissors must be used.

4.6.2.6. No further biopsy can be performed.

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.
Place tissue sample into a collection tube.

Minimize the amount of tissue sample in annex.

Requires identification as per Rodent Identification SOP.

Blood sampling:

- Tail sampling biopsy
- Tail biopsy should only be performed twice over the lifetime of the animal.
- Requires General anesthesia and analgesia are required. Refer to Rat Anesthesia and Rodent Analgesia SOPs.

Skin swabbing:

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

Tail biopsy is ideally performed on rodents before 17 days of age to avoid transaction 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.

Rats are required. Refer to Rat Anesthesia and Rodent Analgesia SOPs.

6.7.1.1. 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.

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

6.7.1.3. Rats must be approximately 7 days old at the time of the biopsy.

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

6.7.1.5. No more than 2 digits (total) can be affected, and only 1 biopsy per paw.

6.7.1.6. Distal phalanx biopsy is acceptable only in the following conditions:

- Sharp iris scissors must be used.

6.7.2.1. Distal phalanx biopsy procedure:

- Sharp iris scissors must be used.

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

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

6.7.2.4. 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.

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

6.7.2.6. Gently, but securely, restrain the rat (manual or mechanical).

6.7.2.7. Place tissue sample into an identified collection tube.

6.7.2.8. 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:

- Apply a drop of tissue glue to tip of tail.
- Apply a chemical cautery agent (e.g. Kwik Stop® powder or silver nitrate stick).
- Electric or heat cauterize the cut end of the tail.

6.7.2.9. Return the rat to its cage.

6.7.3.1. Return the rat to its cage.

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

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.

6.7.8.2. 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.

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

6.7.8.4. 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.

6.7.8.5. Sharply identify animal as per Rodent Identification SOP.

6.7.8.6. Collect blood from the saphenous vein. Refer to toes per SOP 403 Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.7. Identify animal as per Rodent Identification SOP.

6.7.8.8. Collect blood from the tail. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.9. Collect blood from the pinna. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.10. Collect blood from the footpads. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.11. Collect blood from the ear. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.12. Collect blood from the coronary sinus. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.13. Collect blood from the heart. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.14. Collect blood from the aorta. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.15. Collect blood from the femoral vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.16. Collect blood from the popliteal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.17. Collect blood from the jugular vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.18. Collect blood from the jugular vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.19. Collect blood from the femoral artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.20. Collect blood from the carotid artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.21. Collect blood from the aortic arch. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.22. Collect blood from the descending aorta. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.23. Collect blood from the ascending aorta. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.24. Collect blood from the pulmonary artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.25. Collect blood from the pulmonary vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.26. Collect blood from the superior vena cava. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.27. Collect blood from the inferior vena cava. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.28. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.29. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.30. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.31. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.32. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.33. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.34. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.35. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.36. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.37. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.38. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.39. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.40. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.41. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.42. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.43. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.44. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.45. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.46. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.47. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.48. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.49. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.50. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.51. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.52. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.53. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.54. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.55. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.56. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.57. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.58. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.59. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.60. Collect blood from the hepatic vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.61. Collect blood from the portal vein. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.

6.7.8.62. Collect blood from the hepatic artery. Refer to Guidelines for Blood Collection Volumes and Frequency SOP.
Sample Ear Notching Charts

Animal is facing you.

1. One notch on left ear
2. One notch on right ear
3. Notch on left and right ear
4. No ear punch

Right

Left