Skin swabbing:

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

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.

Restrain the animal.

Using a sterile cotton-tipped swab, stroke the ventral and dorsal skin against the direction of hair growth.

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.

Place tissue sample into an identified collection tube.

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

Tail snipping procedure for rats over 21 days of age:

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.

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.

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

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

Distal phalanx biopsy:

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

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

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

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).

Picazo MG, García-Olmo DC. “DNA from tissues of young mice is optimal for genotyping”.

2022.07.12	**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 sampling 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.**
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.** 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**. 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.** 5.4.3. Ensure the ear punch apparatus ~~or scissors are sharp~~ **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. ~~Alternatively, use scissors to make small notches in the ears.~~ See sample in annex.
2022.07.12	5.5. ~~Blood sampling~~ **Whole blood**: 5.5.1. Collect ~~blood from the saphenous vein. Refer to~~**as per SOP 403** Guidelines for Blood Collection Volumes and Frequency ~~SOP~~. ~~5.5.2. Identify animal as per Rodent Identification SOP.~~
2022.07.12	5.6. Tail ~~snipping~~ **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 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.** 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.** **5.6.4. Tail biopsy should only be performed once over the lifetime of the animal.** 5.6.6.2. Gently, but securely, restrain the rat ~~(manual or mechanical).~~ ~~5.6.6.3. Swab the tail with antiseptic (e.g. alcohol).~~ 5.6.6.4. 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 sevffal(5 (s)-1s)-10.6he(e)-7 (v)ue sci(5 (s)-1)-26 (t(in)-14.5l)-10.6 i(t)-14.9 (i19.3 (u)-14.4 T2 1 Tf**[**o)-1.5c 0.02 3.034 0 (n)-15 (d)-160.6 ii1975 (s)-12(s)-15 (d13 (itif)2.6
2022.07.12	

Sample Ear Notching Charts