 <p>THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE</p>	<p>UQ Animal Ethics Committee - Standard Operating Procedure LAB_038 Tissue Collection - Toeing in Mice and Rats Institutional author: UQ Biological Resources AEC Reviewed & Approved: April 2024</p>	Version #5
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LAB_038 Tissue Collection - Toeing in Mice and Rats (Expires Dec 2024)

I. OBJECTIVE

To describe the toeing technique used in mice and rats within UQBR facilities.

NB: The use of (*) indicates this statement is dependent on the facility procedures

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II. SAFETY

1. Facility and procedure appropriate PPE use is essential when handling laboratory rodents
2. All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form
 - This procedure has the risk of mouse bite injury – take appropriate care.
 - This procedure has a risk of causing musculoskeletal injury when performed regularly – consider suitable ergonomic design wherever possible

III. EQUIPMENT


- PPE *
Minimum PPE is gloves and gown, additional PPE may be required based on facility or additional risk e.g. working with infectious material.
- Surgery scissors e.g. Iris Scissors
These should be in good working order and sharp to avoid discomfort for the rodent.
- UQBR Standard Identification System diagram
- Disinfectant *
- Tissue collection tubes and tube holder
- Snap lock bags or boxes
- Tissue Identification Slips *
- Forceps
- Lint free wipes

IV. PREPARATION

1. Turn on Change station or Biosafety Cabinet *
2. Wipe surfaces with disinfectant
This includes disinfecting tools – wiping thoroughly to remove any residue

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IV. PROCEDURE

Administration

Refer to individual facility workflows for administrative requirements

Refer to the animal management database workflows for database requirements

Restraint of Rodents

Refer to LAB_006 Handling and Restraint of Mice

Clean Technique

Use a clean technique when performing this procedure, this will minimise contamination from pathogens and subsequently infection in research animals.

Toeing Procedure

- Ensure all instruments to be used have been disinfected and organic material removed between animals to prevent cross contamination.
- Note that this technique can only be completed in rats and mice between the ages of 6-14 days. UQBR recommends toeing between P10-P12 due to optimal size and incomplete bone ossification.
- These instructions assume tissue is being collected for genotyping purposes. If toeing is completed for identification purposes only, tissue does not need to be collected and other less invasive procedures should be used.
- *The Code* states 3.3.6 Methods used to identify animals must be (i) appropriate for the species and the circumstances (ii) be compatible with the purpose and aims for the project or activity (iii) involve non-invasive methods whenever possible. The use of invasive methods must conform with Clause 3.3.1 (iv) cause the least harm, including pain and distress, to the animals.
- UQBR may collect one additional tissue sample one per rodent in the unlikely event genotype results may need to be clarified, however if such requests are on-going specific AEC approval will be required. Animals that are usually toed would be ear notched in this scenario.

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1. Differentiate females and males. Refer to SOP Sexing Rodents

For identification purposes the numbers are consecutively grouped according to sex, the neonates are placed in a clean container to keep the sexes separated.



Figure 1. Females and males are separated into clean containers (UQBR 2020).

2. Assign identification numbers to the litter starting with females

The animal management database used at UQ is configured will create female ID's first.

3. Label tissue collection tubes

4. Starting with females, restrain the neonate, holding the foot to be toed in one hand

5. Place the toe into the correct position within the scissors above the collection tube

This will ensure the small tissue sample falls into the collection tube without contamination.

Consider if the number to be toed is in the '10's'. The toe that is removed may change to ensure that no more than one toe per extremity is removed. For example the numbers 16-19 will have one toe per rear foot removed.

6. Swiftly cut the end of the toe from the most distal joint.

Ensure the scissors used are well maintained and sharp. Discard all blunted scissors and replace as necessary. Identification numbers ending in '00' may have a tail tip sample taken instead of a toe sample where genotyping is required. In the event of toeing errors, the animal records must be updated to match the error (additional tissue should not be amputated).

Adverse events should be referred to LAB_022 UQBR Veterinary Care Program.

7. The tissue will land in the collection tube. Seal the collection tube.

If the tissue falls onto the work surface, use disinfected forceps to collect and place into the tissue collection tube.

Note: the tissue sample may fall onto the work surface and be drawn into the Cabinet filter. To prevent losing the tissue sample, complete the work over a tray with raised edges ensuring airflow of the cabinet is not obstructed, or work well inside the work area.

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Figure 2. Toeing using fine tipped scissors (UQBR 2020).

8. Disinfect scissors between animals by dipping into 70% ethanol, making sure to wipe dry *
The scissors are dipped or sprayed with 70% ethanol. The scissors are then wiped dry with clean paper towel. This step is required because sensitive genotyping protocols could detect cells from the previous sample causing an inconclusive or incorrect genotype result.



Figure 3. Ethanol filled container to disinfect equipment (UQBR 2020).

9. Repeat Steps until all females are toed
10. Repeat Steps until all males are toed
11. Place collection tubes and tissue identification slip* into snap lock bag or tissue collection box
Follow the specific packing instructions for the genotyping provider that is used.
12. Place the litter into the home cage and return to the rack
13. Disinfect the holding containers ready for next use
14. Disinfect instruments between litters, making sure to remove all residue
15. Continue from Step 1 for remaining litters to be toed
16. Place zip lock bag/box into designated fridge or collection point
Generally tissues samples are stored in a fridge rather than a freezer to avoid degradation of the cells and to improve use of the tissue for genotyping. When tissue is placed into the freezer there is potential for the water molecules expand and burst the cells degrading the DNA.

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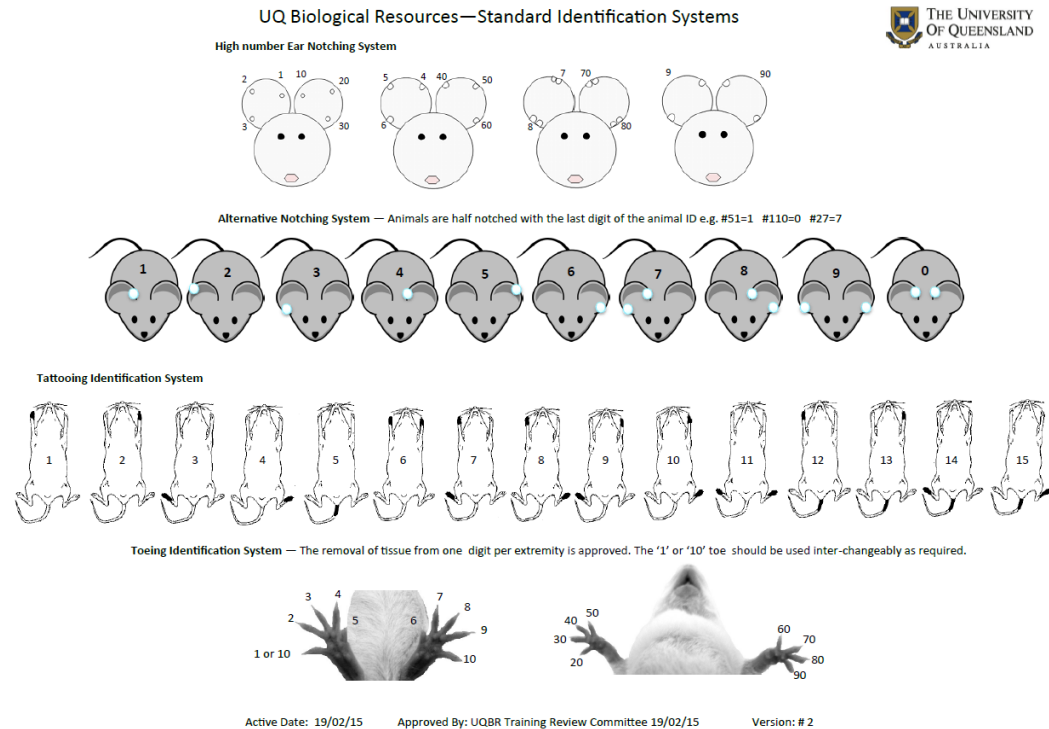


Figure 4. UQBR Standard Identification Systems (UQBR 2020)

Using Alternate Toeing Systems

- It is expected that wherever possible all UQBR Facilities use the UQBR approved standard identification system when toeing. This uniformity ensures all technicians across multiple facilities are familiar with the system. Mice transferred between facilities using the same toe identification system allows easy identification by staff and researchers
- Animal imports will continue to be identified using the external sending facility's method of identification
- Where a lab does not wish to use the UQBR approved standard identification system, approval must be given by the Facility Manager (or UQBR Director in the absence of the Facility Manager)


V. REFERENCE INFORMATION

UQBR Training Consideration

For UQBR training purposes animals may remain for a number of days to monitor. Adverse effects may take time to develop and can assist with the assessment of competency.

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VI. REFERENCES

1. National Health and Medical Research Council (NHMRC) 2008, *Guidelines to promote the wellbeing of animals used for scientific purpose*, viewed 11 April 2019, <https://www.nhmrc.gov.au/about-us/publications/guidelines-promote-wellbeing-animals-usedscientific-purposes>
2. Office of the Gene Technology Regulator (OGTR) n.d., viewed 11 April 2019, <http://www.ogtr.gov.au/>
3. University of Queensland n.d., *Health, safety and wellbeing*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing>
4. University of Queensland n.d., *Incidents, injuries and hazard*, viewed 11 April 2019, <https://staff.uq.edu.au/information-and-services/health-safety-wellbeing/health-safetyworkplace/incidents-injuries-hazards>
5. UQ Biological Resources n.d., *UQBR SOP's*, viewed 11 April 2019, <https://biologicalresources.uq.edu.au/secure/reference-information#SOP's>
6. UQ Biological Resources, 2020 *UQBR Photo Library*.

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