LAB_038 Tissue Collection - Toeing in Mice and Rats (Expiry: December 2027)

I. OBJECTIVE

To describe the toeing procedure used to both identify individual animals and supply tissue for genotyping purposes for UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR.

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

II. COMMENTS / RECOMMENDATIONS -

III. SAFETY AND COMPLIANCE

IV. TRAINING CONSIDERATIONS

Refer to LAB_104 Identification and Tissue Collection Methods in Mice and Rats

V. 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 rodent discomfort.*
- UQBR-REF-011 Standard Identification Systems.
- Disinfectant *
- Tissue collection tubes and tube holder
- Snap lock bags or boxes
- Forceps
- Lint free wipes
- Tray with raised edges

PREPARATION OF EQUIPMENT - Refer to LAB_104 Identification and Tissue Collection Methods in Mice and Rats

IV. PROCEDURE

1. Ensure neonates are between P5-7 to complete this procedure
2. Differentiate females and males.
   For identification purposes the numbers are consecutively grouped according to sex, the neonates are placed in a clean container to keep the sexes separated.

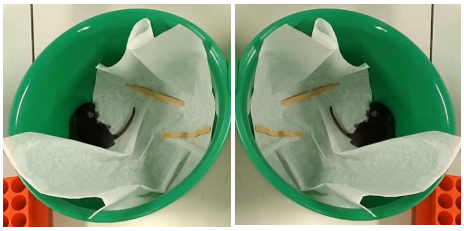

Figure 1. Females and males are separated into clean containers (UQBR 2020).

3. Assign identification numbers to the litter starting with females.
   The animal management database used at UQ is configured will create female ID's first

4. Label tissue collection tubes.
5. Starting with females, restrain the neonate, holding the foot to be toed in one hand.

6. 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 paw is removed. For example, the numbers 16-19 will have one toe per rear foot removed. The number 11 would have the 10 and 1 toe removed.

7. Swiftly cut the most distal phalanx from the toe, that is only remove the distal (3rd) phalanx of the digit at the joint between the 2nd and 3rd bones/phalanges.
   *In the event of toeing errors, the animal records must be updated to match the error (**additional tissue should not be amputated).***

   7a. In the unlikely event a tissue sample is dropped and subsequently lost during the collection process i.e. change station air curtain may blow small samples away, a tail tip may be taken from the tail. Swiftly incise the tip of the tail (maximum 1-2 mm).

8. 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. 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.

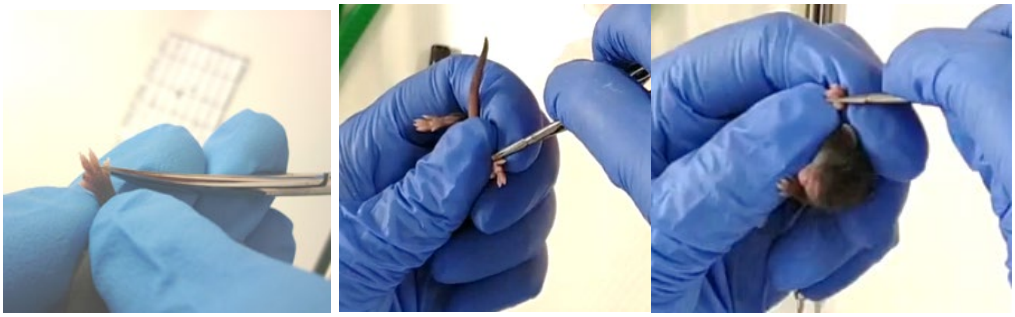

Figure 2. Toeing using fine tipped scissors.

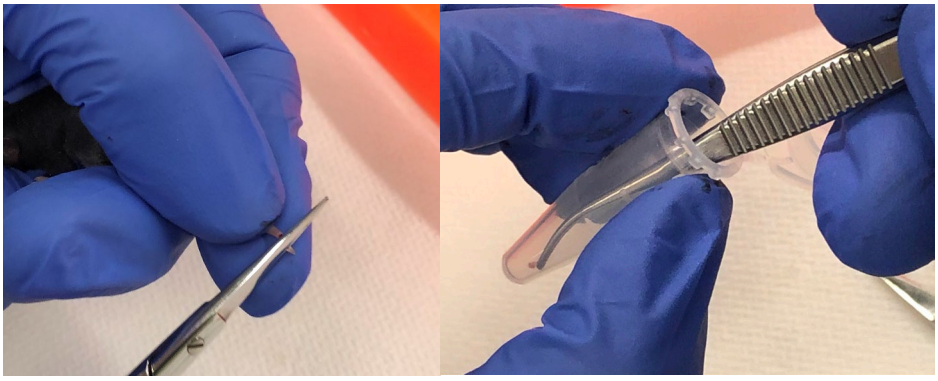

Figure 3. Placement of scissors when performing a tail tip into collection tube.

9. Disinfect scissors between animals by dipping into 70% ethanol, making sure to wipe dry to remove ALL residue *
   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.

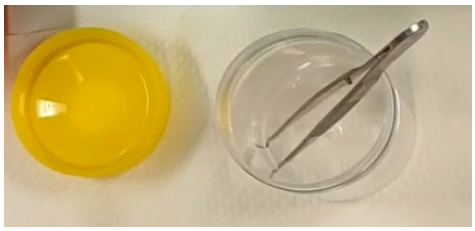

Figure 4. Ethanol filled container to disinfect equipment (UQBR 2020).

10. Repeat Steps until all females are toed and then 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. Finalise relevant administrative tasks related to this procedure
    Examples includes submission of genotyping forms, emailing research group tissue available for collection, recording information on zip lock bags.

17. 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.

18. Monitor pups for bleeding or any distress.

VI. **BIBLIOGRAPHY** Refer to LAB_104 Identification and Tissue Collection Methods in Mice and Rats

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
[#]		[DD/MM/YYYY]	