LAB_024 Tissue Collection – Ear Notching in Mice and Rats

I. OBJECTIVE

To describe the procedure for ear notching of mice and rats used within UQBR facilities.

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. 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.*
- Appropriate facility ear punch
  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
- Change station or Bio-safety cabinet *
- Lint free wipes

IV. PREPARATION

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

V. 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.

Ear Notching Procedure

- Ensure all instruments to be used have been disinfected and organic material removed between animals to prevent cross contamination.
- Note UQBR do not ear notch rodents younger than 2.5 weeks old (17 days) old due to size of the ear and the potential for the ear notch position to be slightly altered.
- 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.

1. Differentiate females and males. Refer to SOP 23 Sexing Rodents
   For identification purposes the numbers are consecutively grouped according to sex, the neonates are placed in a clean container or a separate clean cage to keep the sexes separated.

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 using the scruff technique to avoid head movement
   Use a firm but gentle grip keep the head steady during the procedure.

5. Place the ear notch into the correct position (notch # dependent) within the notch device and above a clean container. See UQBR approved diagram below. *This will ensure the small tissue sample falls into the container and removes the risk of losing the sample. Take care to be in the correct position, avoid notching too close to the head where the cartilage is thicker.*

Figure 1. Placement of the notching device above a clean container (UQBR 2020)

6. Swiftly engage the mechanism to perform ear notch
   Ensure the ear notches are well maintained and sharp. Discard all blunted devices and replace as necessary to avoid potential pain and discomfort, e.g. the device should cut the tissue consistently and reliably.
   In the event of notching errors, the animal records must be updated to match the error (additional notches should not be made).Adverse events should be referred to LAB_022 UQBR Veterinary Care Program.

7. Collect tissue from the ear punch or container and place into the correct collection tube and then seal closed.
   Use disinfected forceps to remove the tissue if it is resting on the ear or the notching device.
   If the tissue is still attached to the ear do not pull with forceps as this can tear the ear tissue. Instead re-notch the same area or use scissors to carefully remove.

Figure 2. Using forceps to remove tissue resting on the fur (UQBR 2020)

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.

8. Disinfect ear punch, forceps and/or scissors wiping thoroughly to remove any residue*
   The ear punch and forceps may be dipped or spray with 70% ethanol. They are then wiped dry with clean paper towel. This step is necessary 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 ear notched
10. Repeat steps until all males are ear notched
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 ear notched
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.

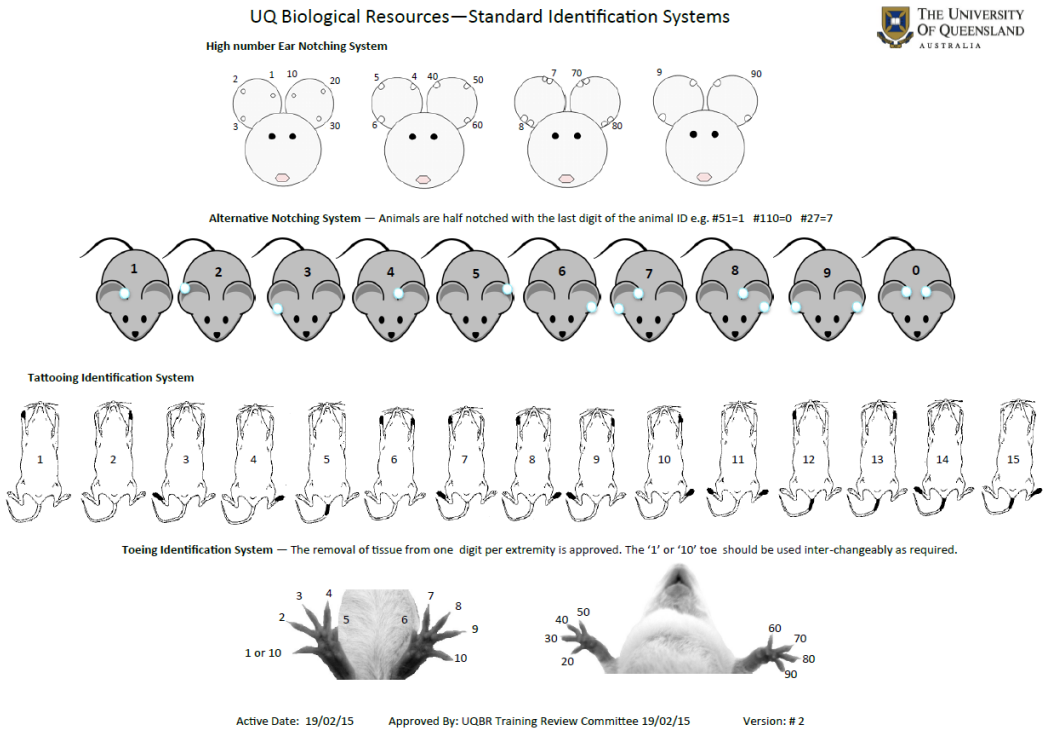

Figure 4. UQBR Standard Identification Systems (UQBR 2020)

Using Alternate Ear Notch Systems

- It is expected that wherever possible all UQBR Facilities use the UQBR approved standard identification system when ear notching. This uniformity ensures all technicians across multiple facilities are familiar with the system. Mice transferred between facilities using the same ear notch identification system allows easy identification by staff and researchers
- Animal imports will continue to be identified using the external sending facilities preferred 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)

VI. 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.

VII. 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*.