# LAB_104 Identification and Tissue Collection Methods in Mice and Rats (Expiry April 2028)

## I. OBJECTIVE

To describe the standard procedures of tissue collection used for the purposes of genotyping and identification of mice and rats used across UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR. To reflect critical information relevant to a technique that is not part of the procedural process.

**NOTE**

- **When citing this SOP**
  - **You must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)**
  - **You must also cite the relevant SOP for the specific technique**
- **The use of (*) indicates this statement is dependent on the facility procedures**
- **The use of (**) indicates this statement is dependent on AEC Approvals**

## II. DEFINITIONS

**Aseptic technique –** Practices employed to protect against introducing infection.

**Clean technique –** Practices employed to prevent or reduce the risk of contamination and overall number of microorganisms from:

- DNA to sample
- Environment to sample
- Cross-contamination between samples
- The site of tissue trauma where tissue collection occurred

**Competent -** "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments."[1]

**Disinfect –** To clean, generally with a chemical to kill bacteria or other small living organisms that cause disease.

**Identification –** The process to apply a marking to a rodent to allow it to be differentiated from other rodents in a cage.

**Genotyping –** The process of determining differences in the genetic make-up (genotype) of a rodent by examining the DNA sequence.

[1] NHMRC, 2013, *Australian code for the care and use of animals for scientific purposes*, National Health and Medical Research Council (NHMRC).

## III. COMMENTS / RECOMMENDATIONS

- These instructions assume tissue is being collected for genotyping or identification purposes.
- *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.
- This SOP includes the below methods of identification and tissue collection for developmental stages as listed in the below table

| Methods of identification and tissue collection for developmental stages | | | |
|---|---|---|---|
| **Procedure** | **Age or Weight** | **Identifies rodent** | **Provides genotyping tissue** |
| **LAB_024 Tissue Collection – Ear Notching in Mice and Rats** | > P17 | ✓ | ✓ |
| **LAB_038 Tissue Collection – Toeing in Mice and Rats** | P5-P7 | ✓ | ✓ |
| **LAB_105 Tattooing – Using the Labstamp Machine in Mice** | >10g <45g | ✓ | X |
| Specific AEC approval is required perform the procedure outside of these approved limitations. | | | |

## IV. SAFETY AND COMPLIANCE

- Possible risks include mouse bite injury, musculoskeletal injury and psychosocial harm.
- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken and risk assessments have been performed. If unsure, consult your supervisor.
- Facility safety protocols should be followed.
- UQBR risk assessments relevant to this task include:

| UQSafe Reference | Risk Assessment Name |
|---|---|
| 1128 | PPE Requirements – Exposure to laboratory animal allergens (LAA) |
| 7005 | Manual handling |
| 3657 | Handling and restraint of laboratory animals under 10kgs |
| 10439 | Wellbeing in laboratory animal workers |

## V. TRAINING CONSIDERATIONS

- All animal tissue collection MUST be performed by appropriately trained personnel who:
  - have been deemed to be competent in the procedure.
  - are confident in completing the procedure.
  - OR are under the direct supervision of a person who is competent.

- Training in tissue collection MUST:
  - be completed under appropriate supervision if occurring on live animals
  - include practices in LAB_002 Clean Technique for Laboratory Animal Surgery

- Workers trained by UQBR must complete:
  - Pre-requisite training such as handling and restraint.
    - LAB_006 Handling and Restraint in Mice and Neonates
    - LAB_039 Handling and Restraint in Rats and Neonates
  - Relevant online learning prior to receiving training in tissue collection procedures.

## VI. EQUIPMENT

- Refer to individual SOP for the technique.
- Refer to the Animal Management Database workflows for record keeping practices.
- UQBR-REF-011 Standard Identification Systems.
- Genetic Research Services submission form (Applies to research groups that use GRS genotyping service).
- Disinfectant - Chlorine dioxide or ethanol
- Sharps instruments such as scissors, ear notches – these are discarded and replaced as required

## VII. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work.
- Check cage cards, animal records and identification to ensure the correct animals are identified.

## VIII. TECHNIQUE CONSIDERATIONS

**Applying clean technique principles to this procedure**

- Sharps instruments and containers used to hold litters will be disinfected:
  - Prior to use
  - Between all litters
  - Following use
- Sharps instruments and containers used to hold litters will be disinfected by:
  - Spraying with Chlorine dioxide (200ppm) or Virkon (1%) and then wipe with clean paper towel to remove residue and organic material.
  - Items are then sprayed with 70% ethanol to remove any remaining chlorine dioxide residue and wiped with clean paper towel.
- Sharps instruments will be soaked using a small container with chlorine dioxide or Virkon then wiped between litters.
- Sharps instruments will be dunked into a small container with 70% ethanol then wiped clean between animals.
- This cleaning routine should be reviewed regularly to ensure that it's appropriate decontaminating instruments between animals for genotyping.

**Avoiding tissue resampling requests (3R's Refinement)**

- Re-sampling animals must be avoided where possible. In the unlikely event that genotype results need to be clarified, UQBR technicians may collect one additional tissue sample per rodent via a half ear notch to avoid anaesthesia.
- Specific AEC approval is required where additional sampling is commonly requested.
- To avoid additional tissue collection and therefore minimise impacts to the animals:
  - Where a second tissue sample is taken due to the numbering system, UQBR will provide both tissue samples so research groups can save this as a back-up sample.
  - Genotyping should be completed as soon as possible to ensure back-up samples can be used if required to avoid challenges in extracting DNA.
  - Research groups should review in-house genotyping protocols and ensure worker training is appropriate to optimise protocols.
  - Consider using UQ's professional genotyping service at Genetic Research Services as a standard, or to assist with genotyping samples that may have been stored.

**Applying Identification Systems**

- UQBR apply the UQBR-REF-011 Standard Identification Systems across all facilities.
- This uniformity ensures all technicians are familiar with the system, this is useful where rodents are transferred across many facilities. This will also support streamlined identification and interpretation across both research groups and technicians.
- Animal imports will reflect the sending facilities identification method.
- Where a lab does not wish to use the standard system, approval must be given by the Facility Manager.
- Research groups should review the identification methods used to ensure it reflects current best practice.

**Handling Neonates**

- Handling neonate can change their smell, where possible transfer smells from the home cage to the pups to minimise the risk of litter or pup abandonment by:
    - Encourage mothers to mark pups.
    - Rubbing dirty bedding or nesting from the home cage onto the litter when placing back into a cage

**LAB_024 Tissue Collection – Ear Notching in Mice and Rats**

- 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 due to growth and development.

**LAB_038 Tissue Collection – Toeing in Mice and Rats**

- Studies in mice indicate that toeing produces no more acute pain or distress than other commonly used rodent identification procedures when performed from 5-7 days of age. UQBR performs toeing from 5-7 days of age where possible, only toeing at 8 days of age where weekends interfere. This reflects the earliest days possible where the neonate is of an appropriate size, webbing has separated between the toes, and bone ossification is incomplete.
- Toes must be completely separated and no longer webbed to perform toeing.
- Only the most distal phalanx is removed from the toe, that is only remove the distal ($3^{rd}$) phalanx of the digit at the joint between the $2^{nd}$ and $3^{rd}$ bones/phalanges. This is technically very difficult but no more than the $2^{nd}$ distal phalanx can be removed.
- No more than 1 toe per paw will be removed.
    - It is important workers apply the UQBR-REF-011 Standard Identification Systems correctly when toeing. For example, when toeing #19, the rodents right hind toe ‘9’ and the left hind ‘10’ are toed, this avoids two toes being removed from the one paw.
- Research groups should consider if toeing may impact their research, behavioural studies that analyse mobility (including grip strength tests, rotorod, treadmill etc) may be impacted particularly if a ‘dew claw’ is removed.
- A tail tip may be taken in the unlikely event a sample is lost during the collection process
    - Tail tip must only be taken on conscious mice and rats less than 21 days old.

**LAB_044 Injections – Tattooing in Neonates**

- This technique requires a clean technique
- No more than 1-2uL is injected
- The tattoo should be no wider than 2mm, excessive pressure on the plunger during injection may cause ink to disperse across the entire paw.
- Low volume syringes are used to improve volume accuracy, e.g. 1mL insulin syringe or a standard 1mL syringe.
- Remove any ink residue with a tissue to avoid possible ingestion by the mother or littermates

**LAB_105 Tattooing – Using the Labstamp Machine in Mice**

- A contraindication for the use of the Labstamp machine is tail defects, this including kinks, bends, wounds, or scars in the tattoo area. The tail must be in normal healthy condition.

## IX. BIBLIOGRAPHY

1. National Institute of Health (NIH) 2022, *Guidelines for Tissue Collection for Genotyping of Mice and Rats* https://oacu.oir.nih.gov/system/files/media/file/2022-01/b3-rodent_genotyping.pdf

2. NHMRC. (2008). Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes: The Assessment and Alleviation of Pain and Distress in Research Animals. National Health and Medical Research Council (NHMRC).

3. NHMRC. (2013). Australian code for the care and use of animals for scientific purposes, 8th edition. National Health and Medical Research Council (NHMRC).

| Version # | Reviewing AEC<br>(note: all other relevant AECs ratify the approval) | AEC Review Date | Version changes |
|---|---|---|---|
| 1.0 | Health Sciences AEC | July 2024 | None |
| 1.0 | Molecular Biosciences AEC | Dec 2024 | None |
| 1.0 | Laboratory Biomedicine AEC | Feb 2025 | None |
| 1.1 | Anatomical Biosciences AEC | April 2025 | Minor administrative |