

THE UNIVERSITY OF HONG KONG

CULATR Policy on Mouse Identification and Genotyping

Wherever animals are kept or used for scientific purposes, the objectives of minimizing any pain or distress they may suffer and promoting high standards of welfare should be as important as achieving the experimental results.

The least invasive method of tissue sampling should be used and the amount of tissues taken should be kept to an absolute minimum. The method chosen for tissue biopsy should not be justified on the basis that it has historically always been used. All procedures must be performed aseptically with proper equipment that is well maintained⁽¹⁻³⁾

Techniques should be regularly reviewed to take advantage of any advance in scientific techniques that allow smaller biopsy samples to be taken or less invasive procedures to be used. All Research Animal Users who perform the following procedures⁴ on mice should follow the recommendations of this document, unless an exception by CULATR has been approved.

I. Ear punch biopsy

- i. Genotyping by Polymerase Chain Reaction (PCR) is the most common and sensitive method. DNA for PCR analysis can be obtained from ear notches/punches samples (which can also be used for identification purposes). Other non-invasive sources which have been published include the hair (containing hair follicles), faecal pellet (containing intestinal epithelial cells), oral swab (containing oral epithelial cells and lymphocytes), and blood.
- ii. Animals younger than 14 days of age should not have the ears punched since the ears are too small to be marked without causing extensive damage to the ear.
- iii. Ear tags may be acceptable with justifications when ear punching or other methods cannot be performed. This method may cause discomfort to the animal and the tags may fall out, being ripped out or caught on cage enrichments. Animals must not be tagged before they are at least 14 days of age.

II. Tattooing

- i. This is a permanent method of identification with little risk of misidentifying individuals.
- ii. This method can be used on young neonates with the tattoo being placed on the tail, toe or ear.
- iii. The procedure must be performed with a neonate or rodent tattooing system. Before tattoo, disinfect the skin and tattoo needles with 70% alcohol.

III. Toe amputation

Toe amputation is likely to cause pain and may impair the mouse's ability to grip and groom. This method must not be routinely used for identification or as a source of tissue for genotyping except as an absolute last resort with CULATR approval.

- i. Mice up to 7 days old may be toe clipped. Toe amputation performed at this age creates only acute pain which is similar to other identification procedures and therefore analgesia is not needed.
- ii. Only the most distal phalanx of only one toe per paw should be removed and no further biopsies may be performed.
- iii. Avoid clipping toes on forepaws if possible. If the forepaw must be used, avoid cutting the innermost toe (equivalent to the “thumb”) of the animal.
- iv. Aseptically prepare the digit before clipping (i.e. wipe with 70% alcohol and wait for it to dry; Note – Povidone-iodine or chlorhexidine solutions may interfere with DNA analysis)
- v. Use very sharp autoclaved surgical scissors to perform this procedure.
- vi. Bleeding may be stopped by applying gentle pressure with a piece of sterile gauze. Monitor animals continuously until bleeding has stopped.
- vii. CCMR technical / veterinary staff must be contacted promptly if toe does not heal properly or if problem arises.

IV. Tail cutting / snipping:

- i. Tail biopsy should not be the first choice for providing DNA for genotyping. The use of non-invasive and more humane methods should be investigated first.
- ii. Tail biopsy if needed to be performed, with justification to the CULATR, can be performed in mice between 8-17 days old when the tail is less ossified and can be significantly shorter. Tail biopsy at older age is undesirable as most mice will react to tail snip probably due to vertebral maturation / ossification.
- iii. A maximum of 2 mm long biopsy will generate sufficient DNA for multiple PCR reactions.
- iv. Repeated tail biopsies must be avoided.
- v. The tail should be disinfected with 70% ethanol and allowed to dry. The tail tip can be removed using a sharp sterile scalpel blade or a pair of autoclaved sharp surgical scissors. In between animals, the instruments should be cleaned of any blood or biological matter, then sterilized with the hot bead steriliser to avoid cross contamination between biopsy samples.
- vi. Analgesia (0.75% bupivacaine immersion for 30 secs after biopsy or EMLA[®] which is a lidocaine/prilocaine cream applied 30-45 minutes before cutting) is recommended.
- vii. The mice must be monitored to assure haemostasis after they are returned to the cage. After cutting, apply sufficient digital pressure or use chemical haemostatic agents (such as styptic powder) to achieve effective haemostasis.
- viii. A tail snip in mice at 18 days old or older must be accompanied by a search for alternatives. Justification must be provided and approval from the CULATR must be sought. Local (EMLA cream or 0.75% bupivacaine) and systemic analgesic (e.g. buprenorphine 0.05mg/kg via subcutaneous injection before snipping) and general anaesthesia (e.g. use of gaseous anaesthetics such as isoflurane which gives a quicker recovery or injectable anaesthetics) is required.

References:

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3. Animal Identification and marking Systems. <https://animalid.com/>
4. 4 <https://norecopa.no/media/6470/norecopa-toeclip.pdf>
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6. https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b9_toe_clipping.pdf
7. Hankenson et al. Evaluation of Tail Biopsy Collection in Laboratory Mice vertebral Ossification, DNA quantity and acute behavioural Responses. J Am Assoc Lab Anim Sci. 2008 Nov; 48(6): 10-18