

RODENT GENOTYPING

1 Policy statement

The proper identification of transgenic animals in a litter is critical to the efficient pursuit of research and in reducing the number of animals involved in a research project. The method of identification used should be reliable and chosen to minimise stress, or subsequent injury, to the animal. Most often the genotype is determined by analysis of DNA extracted from tissues of young mice. Analysis by the Polymerase Chain Reaction (PCR) requires the least amount of DNA.

2 Definitions

Animal	All live fish, amphibians, reptiles, birds, non-human mammals and cephalopods.
Genotype	The two alleles present at a specific locus in the genome.
Polymerase Chain Reaction (PCR)	A common laboratory technique used during molecular genetic testing to produce many copies of a specific DNA sequence, allowing sufficient quantity for analysis.
Deoxyribonucleic acid (DNA)	A molecule that carries the genetic instructions used in the growth, development, functioning, and reproduction of all known living organisms and many viruses.
Institutional Animal Care and Use Committee (IACUC)	The Committee, constituted by an institution, responsible for the oversight and evaluation of all aspects of the institution's animal care and use programme.
Attending Veterinarian (AV)	A veterinarian employed or contracted by an institution, on a full-time or part-time basis, with relevant training or experience in laboratory animal science and medicine to provide adequate veterinary care to the animals in the research facility of the licensee.
Southern Blot	A molecular biology technique used to detect a specific DNA sequence within a DNA sample.

3 Scope

This policy applies to all rodents covered under NUS Animal Care and Use Programme.

4 Guidelines

4.1 Techniques for DNA sampling

- 4.1.1 DNA for PCR analysis can be obtained by a variety of techniques that vary in terms of the discomfort the animal may experience. In order to refine techniques and minimize pain, discomfort or distress to animals, investigators are required to seriously consider these alternatives and to use the least invasive technique that will meet the requirements for the research study. The techniques include:

- a. ear punches or notches. When animals are marked by ear punches or notches, sufficient tissue can be saved to do the PCR. If the animals are to be marked this way anyway, then this technique adds no additional discomfort to that already experienced by the animals.
 - b. oral swabs. Genotyping via oral swabs produces little or no discomfort to the animals.
 - c. hair bulb samples. It is also possible to genotype animals using hair bulb samples although there are some limitations and drawbacks to this procedure.
 - d. tail-clipping. A more commonly proposed technique in which a small portion of the tail of the animal is clipped off.
- 4.1.2 In some cases, larger amounts of DNA are required for Southern Blot determination of the genotype. The IACUC and Attending Veterinarian have determined that obtaining tissue from a mouse for DNA analysis via tail biopsy is a safe, effective and humane procedure that causes minimal or transient pain and distress when performed properly. DNA prepared from tail biopsies is suitable for analysis by either Southern Blot or PCR.
- 4.1.3 Toe clipping for the purpose of genotyping is not allowed unless there is a strong and scientifically justified rationale.

5 References

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2. Dennis, MB. IACUC Review of Genetic Engineering. *Lab Animal* 2000 Mar; 29(3):34-37.
3. Irwin MH, Moffatt RJ and Pinkert CA. Identification of Transgenic Mice by PCR Analysis of Saliva. *Nat Biotechnol* 1996 Sep;14(9): 1146-8.
4. Schmitteckert EM, Prokop CM and Hedrich HJ. DNA Detection in Hair of Transgenic Mice - A Simple Technique Minimizing the Distress on the Animals. *Laboratory Animals* 1999; 33/4: 385-389.
5. Couse JF, Davis VL, Tally WC and Korach KS. An Improved Method of Genomic DNA Extraction for Screening Transgenic Mice. *National Institute of Environmental Health Sciences, National Institutes of Health. BioTechniques* 1994; 17:1030-1032.
6. Malumbres M, Mangues R, Ferrer N, Lu S and Pellicer A. Isolation of High Molecular Weight DNA for Reliable Genotyping of Transgenic Mice. *BioTechniques* 1997; 22/6:1114- 1119.

6 Related Documents/SOPs

1. [CM SOP 401 Tail Biopsy for Rodent Genotyping](#)
2. [CM SOP 570 Rodent Identification](#)

Revision	Author	IACUC approval date
Last reviewed.	Dr Foo Tet Wei, John	13 August 2013
P208.01. Reviewed to: <ol style="list-style-type: none">1. ensure policies are in compliance with the NACLAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and2. up-to-date with current practices Changes made to include "The method of identification used should be reliable and chosen to minimise stress, or subsequent injury, to the animal".	Dr Chew Li Li IACUC	3 January 2023
P208.02. Revisions to: <ol style="list-style-type: none">1. update policy format.2. update the term "animal" to "rodent" in the Scope section.3. add para 4.1.3.	Dr Chew Li Li Ms Keh Lee Hwei IACUC Designated Reviewers	15 December 2025