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INTEGRATED METHOD FOR MOUSE IDENTIFICATION AND GENOTYPING: AN INNOVATIVE APPROACH COMBINING ANIMAL WELFARE, SCIENTIFIC RELIABILITY, AND REGULATORY COMPLIANCE

Nassim Moula, Luc Duwez, Margaux Merchie, Gaelle Lambert, Lilian Briot, Olivier Nivelles, Pierre Drion

Central Animal Facility, University of Liège, Belgium

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¹Co-first authors

Individual identification of mice is an essential procedure to ensure the success of in vivo research. Identification allows researchers to track each animal throughout the experiment, facilitating result analysis while ensuring adherence to ethical protocols. Several methods are commonly used for this purpose, including metal ear tags, electronic chips, toe clipping in young individuals, plantar or tail tattooing, and ear punching.

However, evaluating mouse welfare in relation to the scientific advantages and disadvantages of various identification methods remains complex. Several publications, including those from FELASA working groups, have addressed this issue. For example, the implantation of ear tags, when performed without proper ear preparation, can lead to inflammation, pain, and fibrotic reactions. These effects may negatively impact animal welfare and compromise the validity of research results.

In addition, genotyping genetically modified mice is a routine procedure used to verify the genetic background of strains through polymerase chain reaction (PCR) amplification. This analysis relies on biopsies taken from various tissues, resulting in different levels of trauma and stress for the animals. The most common methods include ear punching, tail biopsy, hair sampling, and buccal or rectal swabs, each with varying success rates for PCR amplification.

It is also important to note that European regulations classify certain tissue sampling methods, such as tail clipping, as invasive. Consequently, animals subjected to these procedures must be included in statistics on animals used in research, even if they do not directly participate in an experimental protocol. To address these challenges, we propose an innovative approach that integrates both identification and tissue sampling for genotyping into a single procedure. This method involves performing an ear punch (1–2 mm in diameter) for genetic analysis, immediately followed by the placement of an identification tag in the same perforation.

This approach offers several key advantages:

- Reduction of stress: By minimizing repeated handling, as both sampling and identification are performed simultaneously.
- Prevention of inflammation: By utilizing the same site for the ear tag placement, avoiding inflammatory reactions commonly associated with conventional tagging.
- 3. Enhanced animal welfare: By reducing the number of manipulations and the associated anxiety.
- 4. Optimized genotyping: By ensuring high-quality samples for PCR amplification.
- Increased scientific reliability: By providing accurate identification while optimizing tissue sampling for genotyping.
- 6. Regulatory compliance: Mice that are genotyped using ear punch sampling during identification are not counted in annual research animal statistics. In France, 80% of mice genotyped in 2023 underwent an invasive method (tail clipping) and were included in these statistics, even though they were not formally enrolled in an experimental protocol.

In conclusion, integrating identification and genotyping into a combined procedure optimizes both mouse welfare and researchers' scientific requirements while adhering to regulatory standards. This innovative approach represents an ideal compromise between research demands and animal ethics.