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Non-invasive sampling methods for genotyping: Improvements towards the 3Rs principle

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Introduction

The Charles River 3Rs Mission strives to advance science by improving laboratory techniques in line with the 3Rs principle, introduced by Russell and Burch (1959), and focuses on enhancing animal well-being. The 3Rs are: Replacement - avoiding or replacing the use of animals, Reduction – minimizing number of animals and Refinement – minimizing pain, stress and suffering.

In research with genetically modified mice, which involves genotyping using invasive ear or tail biopsies in 92% of cases (Mazlan et al. 2014), there is a growing shift towards non-invasive sampling techniques such as oral swabs, or collecting hair or feces. Our EU Charles River genetic testing laboratory has successfully tested and proven the efficacy of genotyping using oral swabs and hair from rodents, aligning with the 3Rs principle and European regulations. The advantages of using non-invasive methods, like oral swabs and hair instead of biopsies are shown in Figure 1 below.

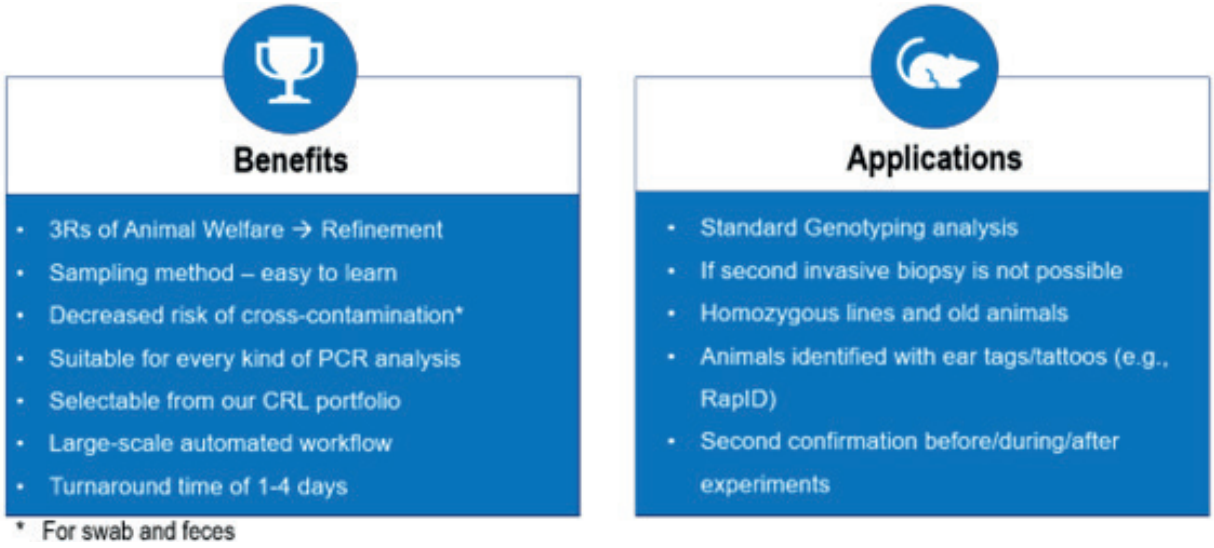

Figure 1. Benefits and applications of non-invasive sampling methods for genotyping

Methods

Housing and husbandry: The genetically modified mice were kept in stable groups within an isolator, under a 12:12-hour light/dark cycle, with temperatures ranging from 20-24°C and humidity levels between 45-65%. The mice were bred and raised under microbiologically defined conditions (specific-pathogen-free (SPF) status according to FELASA standard) and provided with sterilized food pellets and water ad libitum. Cage enrichment included cardboard rodent houses or play tunnels, wooden gnaw sticks and paper tissue. Veterinarians and animal technicians ensured animal welfare daily in accordance with national and international laws and guidelines for the care and use of laboratory animals.

Research Conditions: For the data represented here, both male and female mice from different transgenic lines were used. Due to animal well-being and the size of the swab head, all animals were at least 16 days old before oral swab and hair follicle samples were taken. Several swab-types were tested in terms of the cotton head size and surface structure. Based on the initial testing results, we identified the most effective swab type for achieving optimal genotyping results. A thorough sampling procedure is important to acquire sufficient animal tissue for further processing.

Sampling: Oral swab samples were taken from mice, as shown in Figure 2. The swabs were autoclaved and brought into the animal barrier facility according to standard procedures. Mice were securely scruffed and the swab was twirled around for 5-19 seconds to collect samples from the inside of the cheek. Swabbing was done carefully to avoid hurting the mice. Hair follicle samples were obtained by carefully plucking a small amount of hair (10-20 hairs). After sampling, mice were placed back into their cages. The oral swabs were left to dry before placing each swab and hair sample into individual tubes. Surplus tissue (ear) from individual animal identification was used for routine genotyping and as a control. Finally, the samples were shipped to the genotyping facility. All samples were taken at AAALAC accredited CRL sites according to animal welfare rules and guidelines.

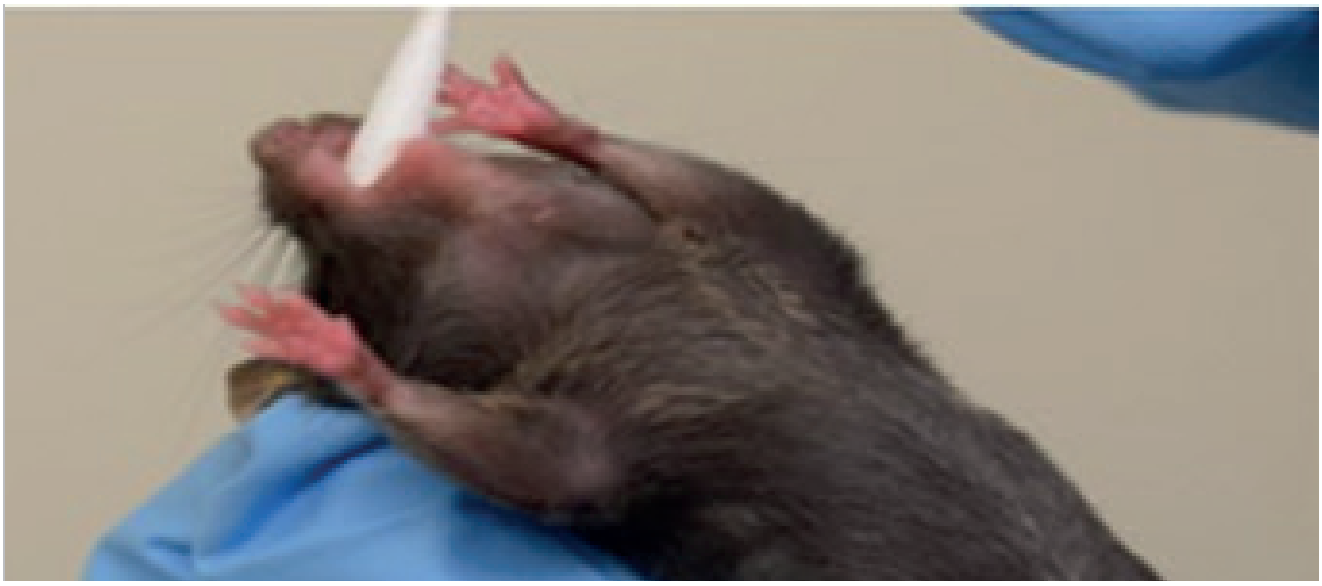

Figure 2. The mouse was securely scruffed to prevent it from moving its head. The autoclaved oral swab was gently inserted at an angle into the oral cavity of the animal to collect the sample from the inside of the cheek

Processing: Throughout the whole processing workflow, samples were kept in a 96-well format to avoid potential mix up and to enable the processing of large number of samples.

Lysis and DNA extraction: The oral swabs were incubated in lysis buffer for 2h at 56°C. Hair and ear biopsies were incubated under the same conditions but overnight. DNA was extracted using Solid Phase Reversible Immobilization (SPRI) technology. Purified DNA was stored at +4°C (short term) until PCR analyses.

Polymerase Chain Reaction (PCR) and analysis of results: DNA extracted from the samples (oral swabs, hair and biopsies) was subjected to either conventional PCR and analysis using capillary gel electrophoresis (CE) (LabChip GX Touch, Perkin Elmer) or real-time PCR (quantitative PCR and endpoint analysis) using StepOne Cycler (ThermoFisher Scientific). Slightly adapted conditions were established if needed e.g. increased number of PCR cycles, template or primer concentration. Results from conventional and real-time PCR were analyzed and compared among the different sample types.

Results

1. Oral swab genotyping - Suitable for every kind of PCR: Oral swabs taken from transgenic lines (KO, KI, etc.) were subjected to conventional PCR with amplicons ranging from 100 to 1500 bp in length (Figure 3A) and to real-time Endpoint analysis (Figure 3B) and zygosity testing (qPCR) for transgenic lines. In our study we could show that >98% of oral swab samples led to clear results. Furthermore, >99% of the results from oral swabs matched those from corresponding ear biopsies.

2. Shipment and Storage conditions for oral swab – (RT (+ 20°C), + 4°C and – 20°C): The robustness of oral swabs genotyping in terms of shipment and storage time/condition were tested for up to 25 days using capillary gel electrophoresis. The percentage ratio of the PCR amplicon in ng/µl for biopsies versus oral swabs is shown in Figure 4. Signals could be detected up to 18 days after sampling if the oral swabs were shipped and stored at RT (+20°C). The best results were obtained when the samples were stored and shipped at -20°C. Visible signals and evaluable results were also detected at +4°C up to 25 days after sampling.

3. Swabs versus Hair: In this study we developed a simple, economic and efficient strategy to extract DNA from hair follicles of mice which are suitable for genotyping. When comparing oral swab and hair follicle samples, we were able to demonstrate consistent genotyping results from hair follicles.

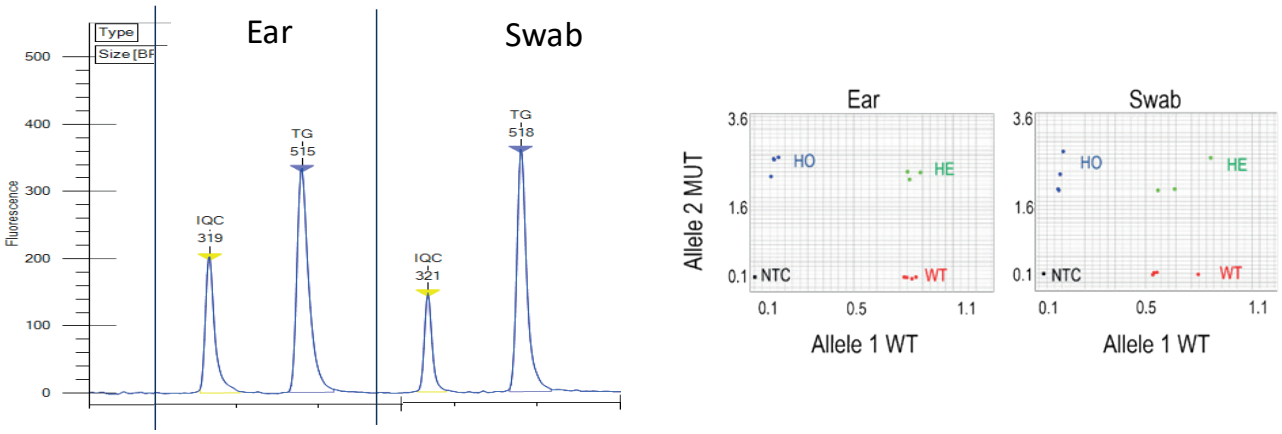

Figure 3. Example electropherogram traces – Amplification products from genomic DNA (ear biopsy or oral swab from the same mouse) up to 520 bp in size). IQC – internal quality control; TG – transgene (A). Allele discrimination plot from ear biopsies (left) and swab samples (right) in endpoint analysis; HO – homozygous; HE – heterozygous; WT – wild type; NTC - negative control samples (B).

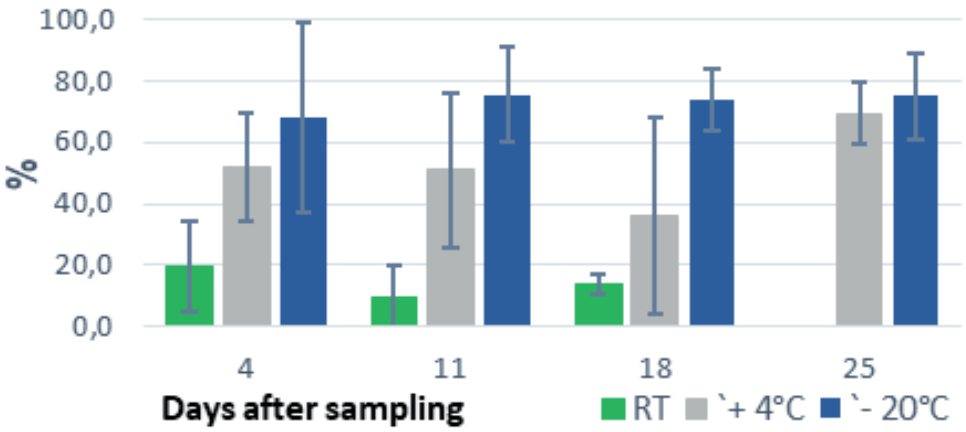

Figure 4. Bar chart. Value was determined from the capillary gel electrophoreses using TG-PCR fragment amplicon amount in ng/µl for biopsies versus oral swabs 4, 11, 18 and 25 days after sampling. Samples were shipped and stored at room temperature (RT: + 20°C), +4°C and -20°C. Standard deviation is shown in the figure. TG – transgene

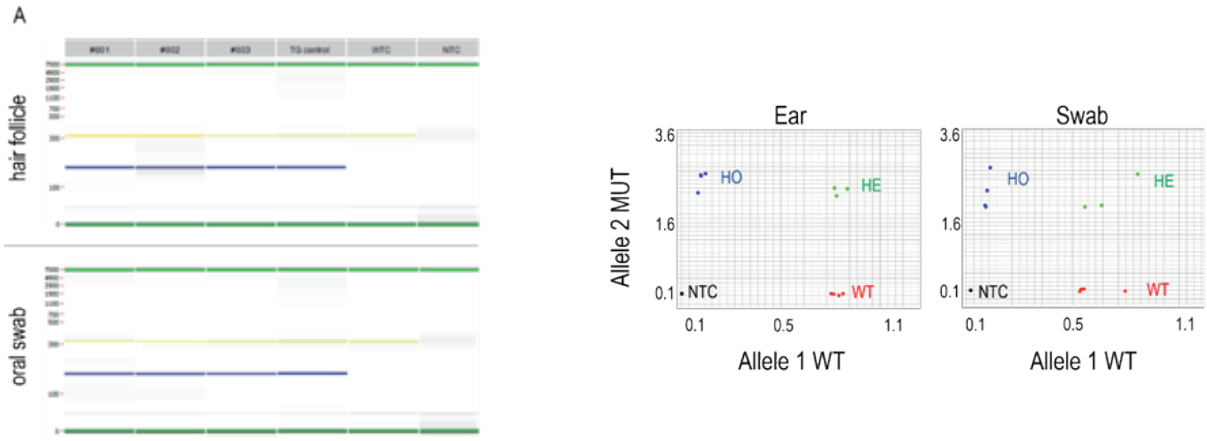

Figure 5. Genotyping using murine hair follicle and oral swab samples. Capillary gel electrophoresis results showing the presence or absence of transgenes in hair follicle and oral swab from the same mouse (A). Example electropherogram traces - amplification products from genomic DNA (oral swab and hair follicle from the same mouse) up to 500 bp in size (B). IQC – internal quality control; TG – transgene (A+B).

Conclusion

In accordance with the 3Rs principle, we have optimized and expanded non-invasive genotyping methods for mice within our automated workflow. This approach involves the collection of oral swabs or hair follicles, and provides an alternative to invasive biopsies, particularly in cases where biopsies are prohibited (e.g., animals with ear tags or tattoos) or a secondary biopsy is not feasible. Non-invasive sampling serves as an appropriate substitute for invasive biopsies, significantly reducing stress and pain. These techniques are applicable to mice aged over 16 days. In addition, these sample types, like standard biopsies, can be processed on a large scale in an automated workflow and are therefore an equivalent alternative for routine genotyping.

References

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