COMPARATIVE ANALYSIS OF DIFFERENT NON-INVASIVE SAMPLING METHODS FOR GENOTYPING: IMPROVEMENT TOWARDS THE 4R PRINCIPLE

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INTRODUCTION

The correct genetic identification of rodents is crucial to the efficiency and reproducibility of research. Moreover, accurate genotyping might help in reducing the number of animals as well as the time and costs associated with research projects. Traditionally, this procedure involves invasive ear or tail biopsies. In 1960, a set of guidelines for the protection of laboratory animals was introduced, known as the 3Rs principle[1] (Replacement, Reduction, Refinement). To extend this principle, Charles River introduced a 4th R - Responsibility. The aim of this principle is to optimize processes and reduce the number of animals needed for research. By consistently using a 96-well format and automating our workflow, we minimize potential errors, allowing us to process large numbers of samples efficiently. Our goal at the EU Charles River genetic testing laboratory has been to find and enhance non-invasive sampling methods that match the efficiency of invasive methods. We have successfully tested and proven the feasibility of genotyping with oral swabs, hair, feces, and dried tears. However, it must be considered that in some countries tears are classified as invasive. The advantages of using non-invasive over invasive methods are shown in Fig.1.

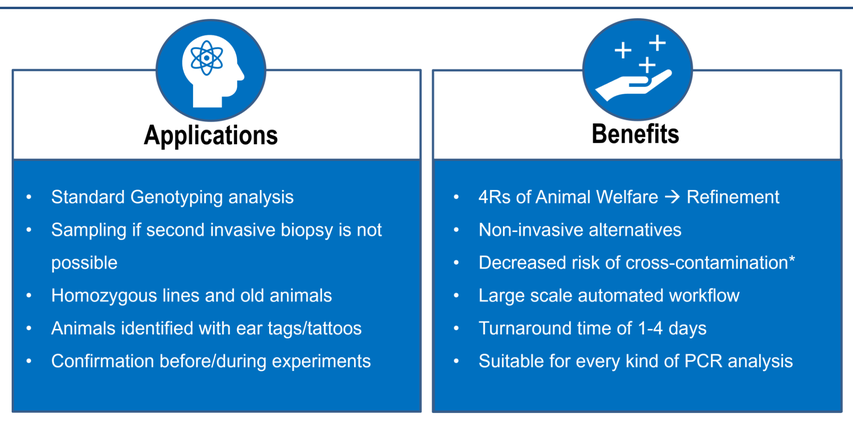

Figure 1: Applications and Benefits of established non-invasive sampling methods for genotyping.
* Not necessarily valid for hair samples

Methods

Research Conditions: . To ensure the welfare of the animals, they were at least 16 days old when the different samples were taken. Oral swabs, hairs, feces and tears were collected from male and female mice of different lines. As controls, ear biopsies taken for routine genotyping analyses from the same animals were used.

Sampling:

- **Oral swabs:** The mice were securely scruffed and the swab was gently twirled around the inside of the cheek for 5-19 seconds.
- **Hair follicles:** The samples were obtained by carefully plucking a small amount of hair (10-20 units).
- **Feces:** For the collection of fecal pellets, the mice were placed into an empty cage, where they normally start defecating within a minute.
- **Tears:** Mice were restrained and the smooth edge of a circular shaped Schirmer tear test paper was inserted into the lower lid margin (inferior fornix) of the eye. This allows the tear fluid to be secreted and absorbed into the test strip from the Meibomian glands.

The oral swabs and tear samples were left to dry before placing each sample into individual tubes.

Shipment and Storage: The samples were shipped to the Genetic Testing Services (GTS) facility on dry ice and stored at -20 ºC.

Lysis and DNA Extraction: The different sample types were all incubated in lysis buffer overnight at 56°C. 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: The DNA extracted from the samples was subjected to either conventional PCR using a capillary gel electrophoresis (CE) (LabChip GX Touch, Perkin Elmer) or real-time PCR (quantitative PCR and endpoint analysis) using a StepOnePlus Cycler (ThermoFisher Scientific). Slightly adapted conditions were established if needed e.g., increased number of PCR cycles, template or primer concentration. Amplification products were analyzed and compared between the different sample types.

Results

1. Conventional PCR: Non-Invasive vs Invasive Sample Types

DNA extracted from the different sample types were run with conventional PCR and analyzed via CE. In our study, we could show that swab, hair, feces and tear samples led to explicit target peaks. Furthermore, the genotyping results obtained from the non-invasive samples matched those from corresponding ear biopsies.

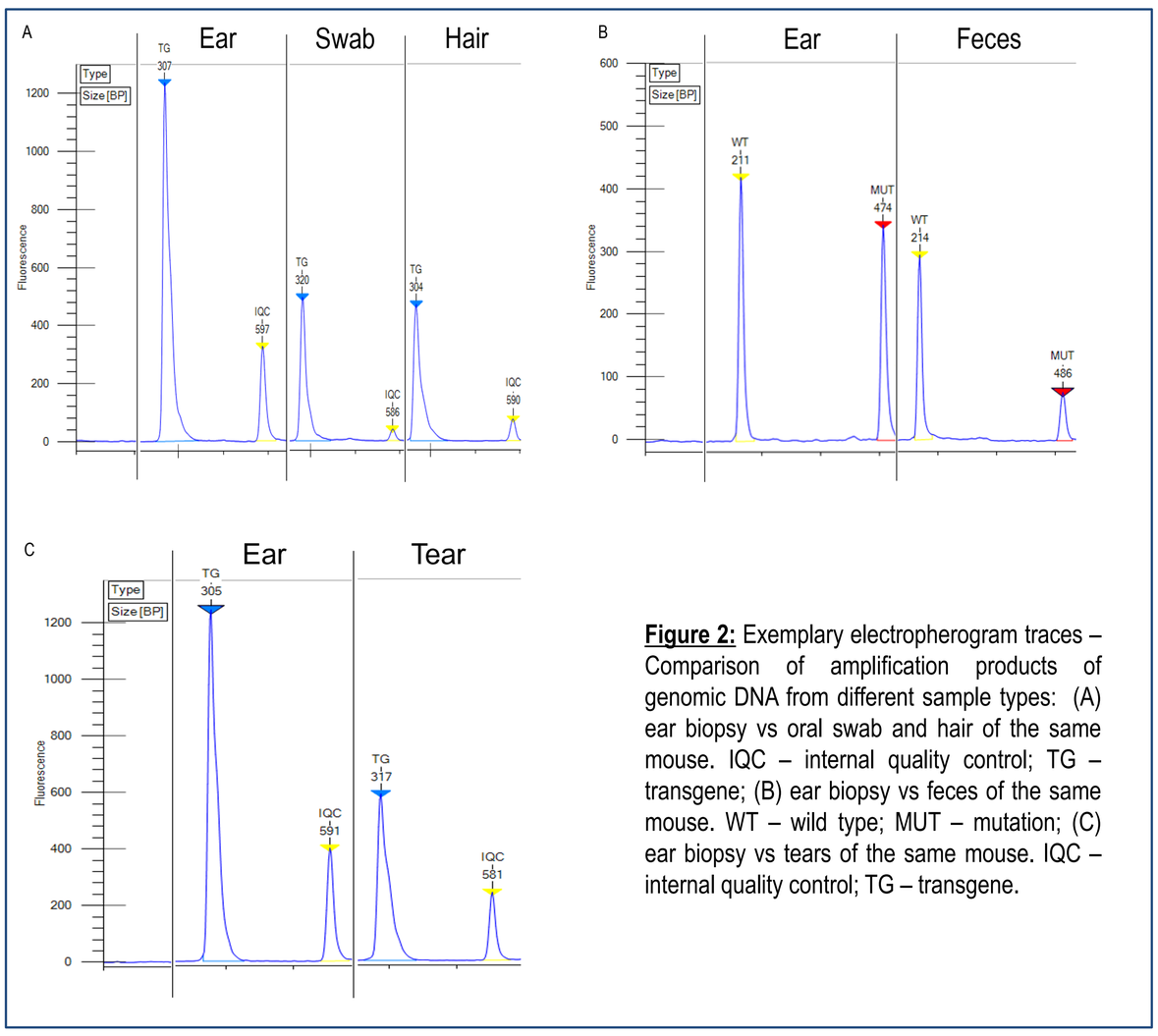

Figure 2: Exemplary electropherogram traces – Comparison of amplification products of genomic DNA from different sample types: (A) ear biopsy vs oral swab and hair of the same mouse. IQC – internal quality control; TG – transgene; (B) ear biopsy vs feces of the same mouse. WT – wild type; MUT – mutation; (C) ear biopsy vs tears of the same mouse. IQC – internal quality control; TG – transgene.

2. Endpoint Analysis: Non-Invasive vs Invasive Sample Types

Real-time Endpoint analysis with DNA extracted from different sample types (allelic discrimination plot (Fig.3)) shows clear signals in the discrimination plot. We could determine that the swab, hair, feces, and tear samples matched the result of their corresponding ear biopsies; thus, showing us accurate results and the correct genotype determination.

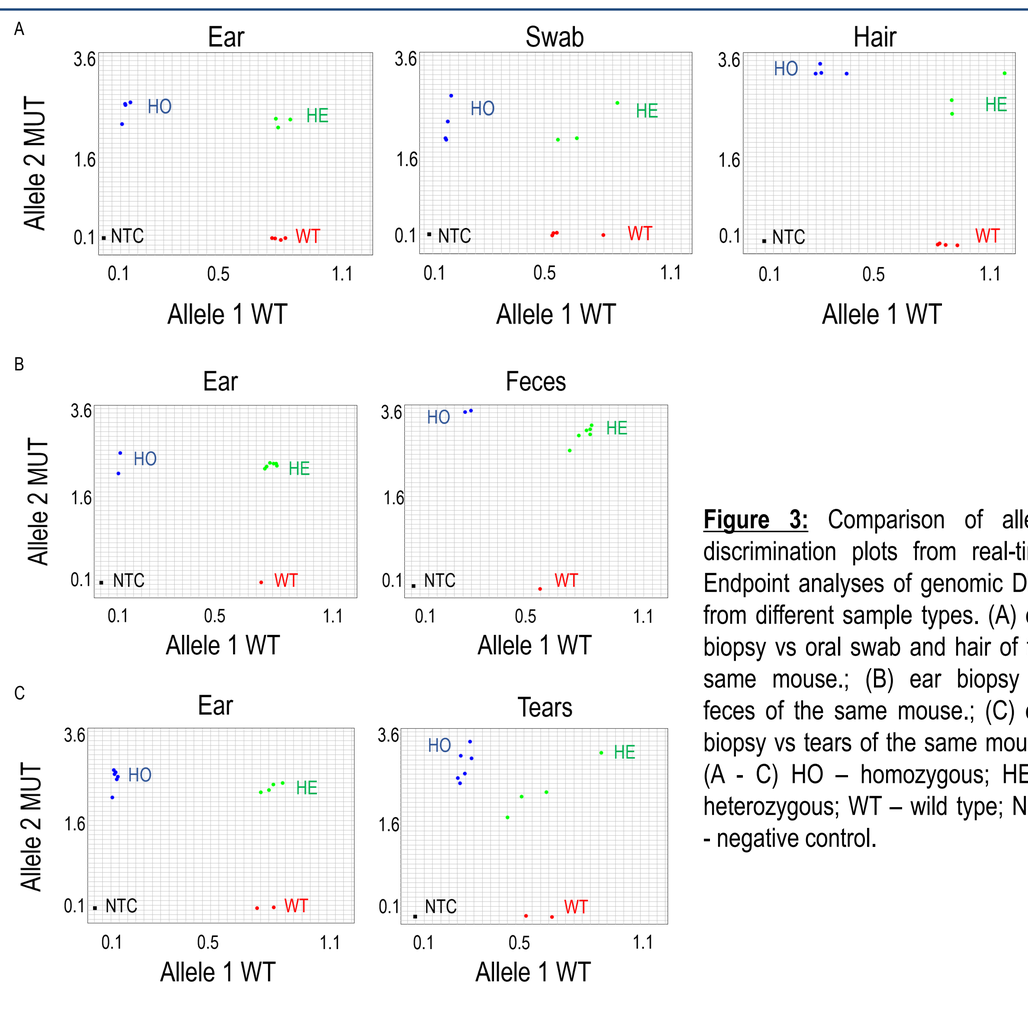

Figure 3: Comparison of allelic discrimination plots from real-time Endpoint analyses of genomic DNA from different sample types. (A) ear biopsy vs oral swab and hair of the same mouse.; (B) ear biopsy vs feces of the same mouse.; (C) ear biopsy vs tears of the same mouse. (A - C) HO – homozygous; HE – heterozygous; WT – wild type; NTC - negative control.

CONCLUSION

Considering the 4Rs and a growing interest in refining genotyping methods, we have achieved our goal by improving four different non-invasive sampling methods that match the efficiency of invasive methods. We have successfully demonstrated the feasibility of genotyping with oral swabs, hair, feces and dried tears.

Non-invasive sampling methods:

- good alternative to invasive biopsies
- replace stressful and painful invasive methods
- can be taken from mice >16 days old
- feces contain more PCR inhibitors
- used for health monitoring (only feces)
- decreased risk of cross-contamination (except hair)

Genotyping using non-invasive sample types:

- sufficient DNA yield although lower
- conventional PCR and real-time Endpoint analysis
- same results as the corresponding invasive samples
- automated, large-scale routine genotyping
- turnaround-time of 1-4 days

REFERENCES

[1] Russell and Burch Methuen & Co. Limited, 1960

ACKNOWLEDGEMENTS

We thank the Genetic Testing Services Erkrath, Germany team for all their work and support. We would also like to thank all our cooperation partners. The project was approved by an ethics committee as per European legislation.