Optimizing Genotyping: A Rapid and Less Invasive Alternative to Tail Clipping in Mice

Joanna Gerges, Makenna Jenkins, Elizabeth A.D. Hammock

[1]Department of Psychology, [2]Neuroscience Program, Florida State University, Tallahassee, FL 32306

Objective

- Traditional DNA extraction methods in mice, particularly the tail clipping method, are time-consuming, invasive, and raise concerns about animal welfare.
- The goal of this study is to evaluate the effectiveness of using sterile toothpicks for DNA sampling in mice. We aim to assess the efficiency and reliability of PCR analysis with this alternative method, across several DNA targets.

Background

Traditional Genotyping in Mice

- Genotyping is a critical step in genetic research, allowing researchers to identify specific genes or transgenes in mice.
- The conventional method involves tail clipping, where a small portion of the tail is removed for DNA extraction.
- This method requires 24–34 hours, including a 24-hour digestion step, before PCR amplification can proceed.
- Tail clipping, though widely used, poses concerns regarding pain, stress, and potential infection in mice, even when performed under anesthesia.

Alternative DNA Sampling Methods

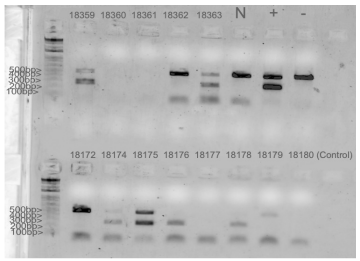
- Researchers have explored less invasive genotyping techniques, such as ear punches, buccal swabs, and fecal sampling, but these methods may not always yield high-quality DNA.
- Forensic studies suggest that toothpicks can effectively collect epithelial cells with sufficient DNA purity for PCR amplification (Rizky et al., 2022).
- Sterile toothpicks have also been used in bacterial colony transfer, allowing direct PCR amplification without DNA extraction, improving specificity and efficiency (Woodman et al., 2018).

Significance of This Study

- This study explores whether sterile bamboo toothpicks can provide a rapid, reliable, and less invasive alternative to tail clipping for genotyping.
- By directly collecting rectal epithelial cells, this approach could:
  - Reduce stress and discomfort in mice.
  - Lower costs by minimizing reagent use and processing steps.
  - Shorten processing time by eliminating the 24-hour digestion step.
  - Ensure consistent and accurate genotyping results comparable to tail clipping.

Figure 1

Gel Electrophoresis of OXTR-CRE



Note. Example gel electrophoresis of PCR products amplifying the Oxtr-cre transgene. Every sample loaded with DNA should produce a PCR product at 500bp. This serves as a DNA loading control. In contrast, only DNA samples from animals carrying a copy of the Oxtr-Cre transgene should have a product at 250bp. While positive (+) and negative (-) DNA controls from tail samples performed as expected, a reaction that did not include DNA (N) showed an unexpected product. Rectal samples from 9/13 mice yielded PCR products, while only 7 out of those 9 were interpretable (showed the expected DNA loading control band at 500bp). These results are promising though additional refinement is needed.

Materials & Methods

Sample Collection

- Sterile tubes were labeled with corresponding numbers.
- Rectal samples were collected using bamboo toothpicks, which were chosen for their high absorbency.
- After collection, toothpicks were placed in their corresponding labeled tubes, spaced apart on a tray.

PCR Protocol Modification

- Reagents were added following the specified genotype protocol.
- PCR protocols were modified by adding 2 µL of Lysis Buffer per sample to account for the lack of template DNA.
- PCR protocol samples were modified by adding 20 µL of reagent mixture to each sample aliquot.

Reagent Application

- Once prepared, the reagent mixture was pipetted into each tube.
  - The pipette was inserted near the tip of the toothpick where the DNA was collected.
  - Toothpicks were swirled 20 times in the mixture in both directions.
  - The toothpicks were soaked in the reagent for one minute before being carefully removed.

Controls

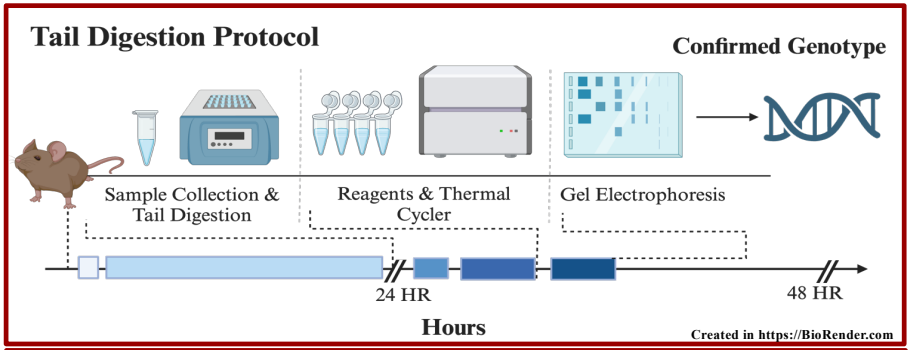
- Control samples were collected from tail digestion samples. Toothpicks were swirled 20 times in each direction and followed the consistent modification/application process.

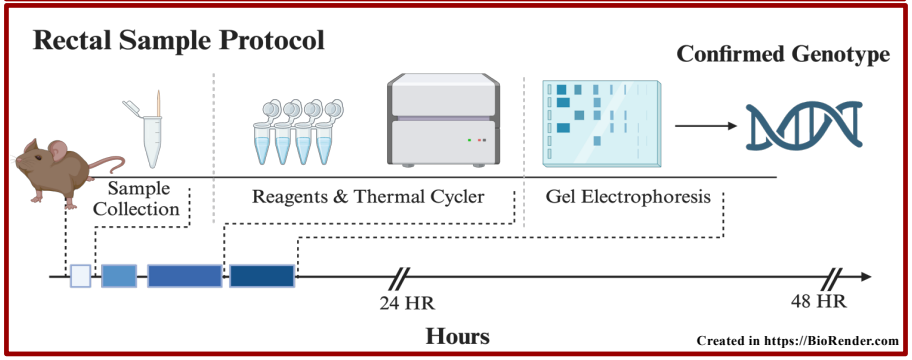
Sample Processing

- This process was repeated for all samples before placing the tubes in the specified thermal cycler protocol.
- After DNA amplification, samples were processed through their specified gel electrophoresis protocol and imaged.

Imaging Comparison

- Rectal sample imaging results were then compared with their tail digestion counterparts to ensure accuracy and effectiveness.





Results & Summary

- Data collection is on-going, though preliminary results suggest high efficiency and accuracy compared to tail digestion.
- Current results align with findings from Rizky et al., (2022) and Meldgaard et al., (2004), cautiously optimistic that refinements will result in improved methods.
- Current focus is on completing further testing to ensure rectal sampling and the modified PCR protocol are easily replicable.
- Additional focus is on confirming that this protocol has strong validity, and can be applied to most target DNA sequences.
- Overall, this protocol saves an average of 24 hours by eliminating the need to digest tail samples.
- Further analysis is needed to confirm consistency and draw final conclusions.

Future Directions

- Conduct additional testing to validate the effectiveness of rectal sampling and the modified PCR protocol across diverse templates.
- Assess result consistency across multiple users to ensure reproducibility.
- Review and refine written instructions for clarity and user-friendliness.
- Optimize DNA extraction and PCR protocols to reduce variability and enhance accuracy and reliability.
- Compare rectal sampling with alternative non-invasive methods to evaluate efficiency and sample validity.
- Explore additional sustainable materials to optimize the DNA extraction and PCR protocols to be more cost-efficient and environmentally friendly.

Acknowledgements

We gratefully acknowledge the support of the Veterinary and Animal Care staff of Florida State University. Research involving the use of live animals was approved under FSU ACUC protocol # 2020-00021.The project described was supported by the Good Nature Institute and UROP. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agency.

References