

Submission ID #: 71206

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21717878>

Title: Skin Swabbing as a Noninvasive Alternative to Fin Clipping for Commercial Automated Genotyping

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? A few floors. One will be an office for the script's speaking aspect. The other will be in the animal unit for the actual procedure.

Current Protocol Length

Number of Steps: 8

Number of Shots: 13

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Mollie Millington:** As an animal technician, my research focuses on improving animal welfare by advancing the principles of the 3Rs- replacement, reduction, and refinement.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Mollie Millington:** This protocol enables zebrafish laboratories that outsource DNA sample processing to refine sample collection while minimizing potential discomfort or harm to the animals.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Mollie Millington:** This protocol allows researchers to collect noninvasive DNA samples suitable for automated genotyping while improving animal welfare.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Mollie Millington:** The most important consideration is to handle the fish gently by swabbing along the direction of the scales and avoiding excessive pressure.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Mollie Millington:** Future research can compare different swab materials and designs to further improve DNA collection and genotyping success rates.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Francis Crick Institute's animal welfare and ethical review board and Home Office licensing

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Skin Swab Sample Collection

Demonstrator: Mollie Millington

2.1. To begin, obtain a plastic container [1] and prepare a workstation containing a soft, wet sponge, a commercially supplied 96-well plate, a swab, scissors for trimming the swab, and a separate isolation tank for the swabbed fish [2-TXT].

2.1.1. WIDE: Talent placing the plastic container on the workbench

2.1.2. Talent arranging all materials in the plastic container **TXT: Wet the sponge using system water only**

2.2. Using a net, transfer the fish from the home tank onto a soft, wet sponge [1]. Gently secure the head using the net and fingers [2].

2.2.1. Talent transferring the fish from the tank onto the wet sponge

2.2.2. Talent securing the head using the net and fingers

2.3. Once the fish is positioned, gently rub the swab along one side of the fish from the pectoral fin to the tail [1-TXT]. Roll the swab by 180 degrees [2], then repeat the swabbing motion 5 more times [3].

2.3.1. Talent gently rubbing the swab from the pectoral fin to the tail of the fish. **TXT: Do not swab against the scales to prevent injury**

2.3.2. CU: Shot of talent rolling the swab 180°

2.3.3. Talent rubbing the swab

2.4. Place the swab in the corresponding well on the plate [1] while transferring the fish to its individual housing [2]. Trim the swab tip with scissors so that it fits inside the well, and allow the plate to dry on the bench for 60 minutes [3]. Once dry, seal the plate with the cap mat [4].

2.4.1. Talent placing the swab into the well of the plate

2.4.2. Talent transferring the fish **NOTE: Shot 2.4.2 was split into two shots. Shot 2.4.2A is a medium shot of the Author moving the fish. Shot 2.4.2B is a close up of the fish being transferred**

2.4.3. Talent trimming the swab tip, so that it fits inside the well, and keeping the plate aside

2.4.4. Talent sealing the plate with the cap mat

2.5. Pack the sealed sample plate for submission to the automated genotyping supplier [5]. After receiving the genotyping results, analyze the data [2].

2.5.1. Talent packing the sealed sample plate for submission to the automated genotyping supplier.

2.5.2. Shot of Talent analyzing the data

Results

3. Results

- 3.1. A total of 470 skin swab samples were processed across nine independent sessions [1]. The protocol achieved an overall genotyping success rate of 86.3% [2]. During Session 1, no genotyping errors were observed [3]. In contrast, Session 4, conducted in a small quarantine room with limited airflow, showed a lower genotyping success rate of 49.3 % [4], with the highest error rate of 36 [5].
 - 3.1.1. LAB MEDIA: Table 1 *Video Editor: Please emphasize the value 470 in the Total Sample column*
 - 3.1.2. LAB MEDIA: Table 1 *Video Editor: Please emphasize the average value 86.3% in the % Success column*
 - 3.1.3. LAB MEDIA: Table 1 *Video Editor: Please emphasize the value 0 in the UD2 and UD4 column for Batch 1*
 - 3.1.4. LAB MEDIA: Table 1 *Video Editor: Please emphasize the value 49.3 % in the % Success column for Batch 4*
 - 3.1.5. LAB MEDIA: Table 1 *Video Editor: Please emphasize the value 36 in the UD2 column for Batch 4*
- 3.2. Excluding Session 4 [1] and following a 60-minute drying step, the overall genotyping success rate increased to 90.9%, demonstrating improved assay performance following protocol optimization [2].
 - 3.2.1. LAB MEDIA: Table 2 *Video Editor: Please emphasize the values 3 and 5 in the Batch column to show the exclusion of session 4*
 - 3.2.2. LAB MEDIA: Table 2 *Video Editor: Please emphasize the value 90.9 % in the % Success column*
- 3.3. Following the introduction of a 60-minute drying step, the average genotyping success rate across Sessions 5 to 9 increased to 90.8% [1]. During Sessions 8 and 9, in which all skin swab samples were collected by the same technician, the average success rate further increased to 92.1% [2].
 - 3.3.1. LAB MEDIA: Table 3 *Video Editor: Please emphasize the value 90.8 % in the % Success column*
 - 3.3.2. LAB MEDIA: Table 4 *Video Editor: Please emphasize the value 92.1 % in the % Success column*

1. Pectoral
Pronunciation link: <https://www.merriam-webster.com/dictionary/pectoral>
IPA: /'pektərəl/
Phonetic Spelling: PEK·tuh·ruhl
2. Genotyping
Pronunciation link: <https://www.merriam-webster.com/dictionary/genotyping>
IPA: /'dʒiːnəˌtaɪpɪŋ/, /'dʒenəˌtaɪpɪŋ/
Phonetic Spelling: JEE·nuh·type·ing, JEN·uh·type·ing
3. Quarantine
Pronunciation link: <https://www.merriam-webster.com/dictionary/quarantine>
IPA: /'kwərənˌtiːn/, /'kwærənˌtiːn/
Phonetic Spelling: KWOR·uhn·teen, KWAHR·uhn·teen