Extraction of DNA from Hair and Keratinized Tissue

Table of Contents

Extraction of DNA from Hair and Keratinized Tissue

1 INTRODUCTION

These procedures describe the process for the cleaning of hair or keratinized tissue (i.e., fingernails) samples in preparation for DNA extraction and for performing the DNA extraction which includes the chemical digestion and purification of deoxyribonucleic acid (DNA).

2 SCOPE

These procedures apply to personnel in the DNA Casework Unit (DCU) that prepare and extract hair or keratinized tissue samples for further DNA testing.

3 EQUIPMENT

3.1 Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, vortex, forceps)
- Magnetic stand
- Stereomicroscope
- Sonicator
- Micro tissue grinder (aka mortar and pestle), if needed

3.2 Reagents

- Xylene and/or xylene substitute, if needed
- Terg-a-zyme, powder or 5% solution
- Ethanol (EtOH), absolute
- Qiagen® Buffer ATL
- Dithiothreitol (DTT), 5M solution
- Proteinase K (ProK), 20mg/mL
- Qiagen® Buffer AL
- PrepFiler® Forensic DNA Extraction Kit
    - PrepFiler® Magnetic Particles
    - PrepFiler® Wash Buffers A and B
    - PrepFiler® Elution Buffer (or TE^{-4} Buffer)
- Isopropanol, 70% Water, reagent grade or equivalent
- Sulfuric acid, 4 N, if needed

4 STANDARDS AND CONTROLS

At least one extraction control (i.e., reagent blank [RB]) must be processed in parallel with each extraction batch starting with digestion.

For evaluation of the extraction controls, refer to the appropriate DNA interpretation procedure.

5 PROCEDURE

Refer to the DNA Procedures Introduction (i.e., BIO-100) for applicable general precautions and cleaning instructions. Ensure the appropriate fields (i.e., instruments, reagents) in STACS™ Casework are completed, as necessary.

Supplies typically needed for 1 sample and 1 RB (adjust for batches):

- EtOH (~1 mL)
- Water (~10mL)
- 5M DTT (~15 µL)
- Buffer ATL (~1 mL)
- Buffer AL (~1 mL)
- Isopropanol (~400 µL)
- Wash Buffer A (~2 mL)
- Wash Buffer B (~1 mL)
- Elution Buffer (~150 µL)
- Magnetic beads (DO NOT UV)
- ProK (DO NOT UV)
- 4 - 1.5 mL tubes (sample, RB, final sample extract, final RB extract)
- Ruler, magnetic stand, forceps, scissors, scalpel, tube rack
- p20, p200, p2000 pipettes

5.1 Sample Collection Guidance

- Except as noted, the following steps are performed in a hood.
- A description of the collected sample will be recorded in the notes.

5.1.1 Hair

- Any step involving manipulations of difficult hairs may occur outside of a hood with the aid of a stereomicroscope.
- Reverse action forceps may aid in grasping a hair.
- **The hood air flow must be off while transferring/handling hair samples.**

1.	View hair under stereomicroscope for presence of root tissue or adherent material.

If sheath material is present, consult an Examiner.

2.	Measure hair and record length. Remove ~ 2 cm of hair from root end (if applicable) and place in a tube.

The sample may be moistened with water to minimize the effects of static.

Longer hair fragments may be processed at the discretion of an examiner.

5.1.2 Fingernail Clipping

1.	Place ~ 3 mm x 3 mm of a fingernail in a tube.

5.2 Sample Cleaning Methods

- The cleaning method(s) performed will be recorded in the notes.
    - In general, at least 1 Terg-a-zyme wash is done for all samples.

- For a sample that will be consumed, typically 2 xylene (or xylene substitute) washes and 2 Terg-a-zyme washes are performed.
- Additional cleaning may be performed at the discretion of an examiner.
- Wash procedures may be repeated using fresh cleaning solutions.
- Smaller rinse tubes and/or less liquid may be used when working with smaller hairs.

- Throughout these procedures, a pulse spin may be done to force a sample to bottom of a tube, as necessary.

- A sample may remain in the same tube for each cleaning step with the removal of the cleaning liquid(s) or a sample may be transferred from one tube of cleaning liquid to the next using forceps.
  - The technique utilized is at the discretion of the biologist based on the size, coloration, and/or condition of the sample.

5.2.1 Xylene Wash (Optional)

When possible, xylene should be handled within a chemical fume hood. (See Safety section).

1.	• Add enough xylene to cover sample. • Sonicate at least 20 minutes in chemical fume hood.

Alternatively, xylene may be added to the collection tube prior to the addition of sample.

A UV-treated xlyene substitute (e.g., xyless) may be used in place of xylene. Xylene substitute does not require sonication within the chemical fume hood.

2.	• Remove xylene and appropriately discard waste. • Add enough reagent grade water to cover sample and mix.

When necessary, the sample may be transferred from the xylene tube to a separate water tube with use of forceps, provided this transfer is NOT done within the chemical fume hood.

Remove the water or transfer the sample to the next tube for additional cleaning.

5.2.2 Terg-a-zyme Wash

1.	• If necessary, prepare a 5% Terg-a-zyme solution for daily use.

5% Terg-a-zyme solution	
Terg-a-zyme	0.5 g
Water	10 mL

2.	• Transfer sample to tube containing enough 5% Terg-a-zyme solution to cover sample. • Sonicate at least 20 minutes.

3.	• Transfer sample to tube containing enough EtOH to cover sample and mix to rinse.

4.	• Transfer sample to tube containing enough water to cover sample and mix to rinse.

Remove the water or transfer the sample to the next tube for additional cleaning or proceed to digestion.

5.3 Digestion

1.	• Prepare the Digestion Buffer.

Digestion Buffer	
5M DTT	13.2 µL
Buffer ATL	1 mL

2.	• Add 300 µl of Digestion Buffer and 20µL ProK to each sample and RB tube. • Ensure samples are submerged. *Samples may be cut into pieces to ensure full immersion.* • Vortex • Incubate tubes at 56°C at 900 rpm for a minimum of ~30 minutes, until the sample is fully digested, or overnight (O/N).

The sample is generally transferred from the last cleaning tube to a new tube containing the digestion buffer; however, the sample may remain in the same tube with the removal of the water rinse prior to the addition of the digestion buffer.

NOTE: For keratinized tissues, a minimum incubation time of 2 hrs is recommended.

If full digestion does not occur after a minimum of 2 hrs, a partially-digested hair sample (and RB) may undergo the grinding process in section 6.5.

3.	• Pulse spin. • Add 300 µL Buffer AL. • Vortex tubes and incubate at 70°C at 900 rpm for 10 minutes.

4.	• Pulse spin and allow to come to room temperature (~5 minutes).

5.4 PrepFiler® DNA Extraction Kit Purification

Prior to addition, vortex PrepFiler® Magnetic Particles tube for 5 seconds until no visible pellet remains in bottom of tube. Pulse spin. *If processing multiple samples, vortex every ~5 minutes.*

1.	• Add 15 µL of Magnetic Particles. • Vortex at **low speed** for 10 seconds. • Pulse spin.

2.	• Add 180 µL of isopropanol. • Vortex at **low speed** for 5 seconds. • Mix at room temperature at 1,000 rpm for 10 minutes in shaker.

3.	• Vortex at **high speed** for 10 seconds. • Pulse spin.

4.	• Place tubes in magnetic stand. • Wait until size of pellet on back of tubes stops increasing (~3 minutes).With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.

5.	• Add 600 µL Wash Buffer A. • Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). • Pulse spin.

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

6.	• Place tubes in magnetic stand for ~60 seconds. • With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.

7.	• Add 300 µL Wash Buffer A. • Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). • Pulse spin.

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

8.	• Place tubes in magnetic stand for ~60 seconds. • With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet.

9.	• Add 300 µL Wash Buffer B. • Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). • Pulse spin.

It is acceptable to have visible aggregates in solution or on side of tube below meniscus.

10.	• Place tubes in magnetic stand for ~60 seconds. • With tubes in magnetic stand, pipette off and discard all liquid without disturbing pellet. • With tubes remaining in magnetic stand, open and air-dry in hood with blower for ~8 minutes.

DO NOT OVERDRY. *If the room temperature is >25°C, reduce the drying time to 5 minutes.*

11.	• Add 65µL of Elution Buffer or TE^{-4}. • Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). • Pulse spin.

If an alternate volume is used for elution, record the volume in the case notes.

12.	• Incubate at 70°C and 900 rpm for 5 minutes. • Vortex at **high speed** until there is no visible pellet on side of tube (~2 seconds). • Pulse spin.

13.	• Place tubes in magnetic stand. • Wait until size of pellet on back of tubes stops increasing (~2 minutes.). • Transfer liquid into final extract tube without disturbing pellet.

If an extract is discolored, spin at ~10,000 X g for 7 minutes and transfer supernatant to new tube.

5.5 Grinding (If necessary)

If necessary, the following procedure may be utilized if full digestion has not occurred after a minimum of 2 hrs.

1.	• Moisten swab with 5% Terg-a-zyme solution. *Terg-a-zyme may be warmed prior to use.* • Scrub mortar and pestle with swab. • Simulate grinding. • Rinse with water. • Repeat two times.

2.	• In chemical fume hood, add 300uL 4 N sulfuric acid to mortar and simulate grinding. • Soak mortar and pestle in 4 N sulfuric acid for 20 minutes.

3.	• Rinse the mortar and pestle with water. • Pulse spin the pestle. • Remove remaining water and crosslink.

4.	• Transfer 200 µl of solution from the RB tube to mortar and simulate grinding. • Remove pestle from mortar. • Transfer liquid back to RB tube.

5.	• Transfer 200 µl of solution and the undigested sample fragments to mortar and grind until fragments are no longer visible. • Remove pestle from mortar. • Transfer liquid back to sample tube.

6.	• Add 20µL ProK and 4µL of 5M DTT to each sample and RB tube. • Vortex and incubate at 56ºC at 900 rpm for a minimum of 30 minutes and a maximum of O/N. • Pulse spin. • Resume digestion processing.

6 LIMITATIONS

- The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

- A hair does that not fully digest may proceed through this procedure without additional grinding.

7 SAFETY

- All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material.
- Refer to the FBI Laboratory Safety Manual for information on personal protection, the proper disposal of the chemicals used in these procedures, as well as the biohazardous wastes generated.
- Procedural Specific Chemical Hazards:
    - Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.
    - Xylene is an irritant and is toxic. Its use should be confined to a chemical fume hood whenever possible.
    - Sulfuric acid is caustic. Gloves, safety glasses, and a laboratory coat must be worn whenever using sulfuric acid. Addition of sulfuric acid to the grinder must be performed inside a chemical fume hood.

8 REVISION HISTORY

Revision	Issued	Changes
00	02/04/2022	Reformatted DNA 402-0 into new template and assigned new Doc ID. Added additional guidance to the sample cleaning methods section. Added appendix A.

9 APPENDIX A: QUALITY CONTROL PROCEDURES

Refer to the DNA procedure for reagent purchasing, preparation and records (i.e., BIO-103) for additional requirements.

A. Contamination check of Prepfiler Extraction Kit

Each new lot of Prepfiler Extraction Kit should be evaluated for the presence of extraneous DNA prior to its use on evidentiary samples. Reagents may be concurrently tested or tested with an in use lot of the counterpart reagent(s).

1. Extract, at a minimum, five reagent blanks (RBs) following sections 5.3 through 5.4 using the new lot of Prepfiler Extraction Kit.
2. Amplify the five reagent blanks, a positive control (HL60), and a negative control (reagent grade water) with a more sensitive region (usually HV1B) for 36 cycles and process through sequencing.
3. Passing Criteria:
    i. A majority of the five RBs and the negative control should result in no sequence data. If the RBs show possible contamination after sequencing and a source can be reasonably explained, the reagent may be put into use.
    ii. The positive control must have the correct sequence.
4. If the contamination check of the Prepfiler Extraction Kit does not meet the above listed criteria, the process will be repeated. If the results are still deemed unsuitable, then the Technical Leader will be consulted.