

# Supplementary Material

## Fragmented Nuclear DNA is the Predominant Genetic Material in Human Shed Hair Shafts

by Brandhagen et al.

### Protocol used to wash and lyse/digest hair shafts.

#### Equipment/Materials

- General laboratory supplies (e.g., tubes, pipettes, vortex, forceps)
- Magnetic stand
- Stereomicroscope (optional)
- Sonicator

#### Reagents

- Xylene and/or xylene substitute, if needed
- Terg-a-zyme, powder or 5% solution (Alconox)
- Ethanol (EtOH), absolute
- Buffer ATL (Qiagen)
- Dithiothreitol (DTT), 5M solution
- Proteinase K (ProK), 20mg/mL
- Buffer AL (Qiagen)
- PrepFiler® Forensic DNA Extraction Kit (ThermoFisher Scientific; cat# 4463351)
  - PrepFiler® Magnetic Particles
  - PrepFiler® Wash Buffers A and B
  - PrepFiler® Elution Buffer (or TE<sup>-4</sup> Buffer)
- Isopropanol, 70% Water, reagent grade or equivalent

Do NOT UV the Magnetic Particles or Proteinase K.

- Any step involving manipulations of difficult hairs may occur outside of hood with aid of stereomicroscope. Reverse action forceps may aid in grasping a hair. The hood air flow must be off while transferring/handling hair samples.
- View hair under stereomicroscope for presence of root tissue or adherent material.
- Measure hair and record length. Remove ~ 2 cm (or more if needed) of hair from root end and place in a tube.  
*The sample may be moistened with water to minimize the effects of static.*

Each wash procedure may be performed additional times using fresh cleaning solution. Pulse spin, as necessary, throughout procedure to force sample to bottom of tube. A sample may remain in the same tube for each cleaning procedure with the removal of the cleaning liquid(s). In instances with smaller samples, smaller rinse tubes and/or less liquid may be used.

### **Xylene Wash (Optional)-Not used in this study.**

- In chemical fume hood, 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.*

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

*As an alternative to the xylene removal, sample may be transferred from xylene tube to a separate water tube with use of tweezers.*

### **Xylene Substitute Wash (Optional)- Not used in this study.**

- Transfer sample to tube containing enough UV-treated xylene substitute to cover sample. Sonicate at least 20 minutes.

### **Terg-a-zyme Wash**

- To make 5% Terg-a-zyme solution, add 0.5 g Terg-a-zyme to 10 mL water.
- Transfer sample to tube containing enough 5% Terg-a-zyme solution to cover sample. Sonicate at least 20 minutes. Repeat once or twice if necessary.
- Transfer sample in a tube containing enough EtOH to cover sample and mix to rinse.
- Transfer sample in a tube containing enough DNA-free water to cover sample and mix to rinse.

### **Lysis/Digestion**

- To make the digestion Buffer: add 13.2  $\mu$ L 5M DTT to 1 mL Buffer ATL.
- Add 300  $\mu$ L of Digestion Buffer and 20 $\mu$ L ProK to each sample and RB tube. Ensure samples are submerged. *Samples may be cut into pieces to ensure full immersion.*
- Vortex and incubate tubes at 56°C at 900 rpm for a minimum of ~30 minutes, until the sample is fully digested, or overnight (O/N).

*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.*

*If full digestion does not occur after a minimum of 2 hours, a partially-digested hair sample (and RB) may undergo the grinding process at the end of this procedure.*

**Purification protocol A:** This protocol currently used by the DNA casework section at the FBI Laboratory. Favors recovery of larger (>100bp) DNA fragments

- Pulse spin tubes with lysate. Add 300 µL Buffer AL. Vortex tubes and incubate at 70°C at 900 rpm for 10 minutes.
- Pulse spin and allow to come to room temperature (~5 minutes).

#### **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.*
- Add 15 µL of Magnetic Particles. Vortex at **low speed** for 10 seconds. Pulse spin.
- 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. Vortex at **high speed** for 10 seconds. Pulse spin.
- **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.**
- 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.*

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

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

- 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.*

- Add 50-65µL of Elution Buffer or TE<sup>-4</sup>. Vortex at **high speed** until there is no visible pellet on side of tube (~5 seconds). Pulse spin.

- 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.
- 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 10,000 x g for 7 minutes and transfer supernatant to new tube.*

**Purification protocol B:** uses a buffer that favors the binding of small (<100bp) DNA fragments to silica.

- To make binding buffer (for 5 samples), mix 16 ml PB buffer (Qiagen), 480 µl sodium acetate (3M) and 40 µl of sodium chloride (5M).
- Transfer the 320 µl lysate solution to a 15 ml conical tube and add 3 ml of binding buffer. Incubate on a flat shaker for at least 1 hour at room temperature.
- Prepare the Qiagen vacuum manifold by adding in order: one VacConnector (Qiagen, cat# 19407), one MinElute column (MinElute PCR purification kit, Qiagen) and one 3 ml extender (Qiagen, cat# 19587). Save the reservoir tubes provided with the columns.
- Transfer the binding buffer+lysate in the extender/column
- Wrap extenders loosely with para film to protect from contamination. Vacuum slowly until the MinElute column is empty.
- Add 3ml PE buffer (Qiagen) to the extender/column and vacuum slowly until the MinElute column is empty.
- Transfer the MinElute column back into the reservoir tube and centrifuge the columns for 1 minute at maximum speed.
- Transfer the column to a DNA free low binding 1.5 ml tube.
- Add 50-100 µl of Qiagen EB buffer or EBT buffer (EB buffer + 0.05% Tween 20) and let it stand for 5 minutes at room temperature.
- Centrifuge the columns for 1 minute at maximum speed.
- Pipette out the EB buffer and transfer it back into the same column. Let it stand for 5 minutes.
- Centrifuge for 1 minute at maximum speed. Remove column, close tube and store extract at -20°C.

**Purification protocol C:** produces two extracts per sample. One containing mostly the large DNA fragments lost Using Protocol B alone and one containing the small DNA fragments that can be lost using protocol A alone.

- Follow protocol A until section highlighted in red
- Place tubes in magnetic stand for ~60 seconds. With tubes in magnetic stand, pipette off and **TRANSFER** all liquid to a 15 ml conical tube, without disturbing pellet instead of discarding it.
- Mix the “liquid waste” with 5 ml of binding buffer described in protocol B.
- Incubate the solution on a flat shaker for at least 1 hour at room temperature.
- During this incubation, finish your first extraction following protocol A (wash the beads with Wash Buffer A, etc..).

→ Results in Extract # 1

2<sup>nd</sup> extract obtained with from the mixing of the liquid waste from protocol A and the binding buffer of protocol B as stated above (Bullet 3, Protocol C)

- Prepare the Qiagen vacuum manifold by adding in order: one VacConnector (Qiagen, cat# 19407), one MinElute column (MinElute PCR purification kit, Qiagen) and one 3 ml extender (Qiagen, cat# 19587). Save the reservoir tubes provided with the columns.
- Transfer the binding buffer + lysate in the extender/column
- Wrap extenders loosely with para film to protect from contamination. Vacuum slowly until the MinElute column is empty.
- Add 3ml PE buffer (Qiagen) to the extender/column and vacuum slowly until the MinElute column is empty.
- Transfer the MinElute column back into the reservoir tube and centrifuge the columns for 1 minute at maximum speed.
- Transfer the column to a DNA free low binding 1.5 ml tube.
- Add 50-100 µl of Qiagen EB buffer or EBT buffer (EB buffer + 0.05% Tween 20) and let it stand for 5 minutes at room temperature.
- Centrifuge the columns for 1 minute at maximum speed.
- Pipette out the EB buffer and transfer it back into the same column. Let it stand for 5 minutes.
- Centrifuge for 1 minute at maximum speed. Remove column, close tube and store extract at -20°C.

→ Results in Extract # 2