

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Extraction of Exogenous DNA from Nails		
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Extraction of Exogenous DNA from Nails

1 Preparation

- 1.1 Extraction sets consist of 10 samples and two Extraction Negatives. Additional extractions may continue sequentially during incubations.
- 1.2 Follow the procedures for Work Place Preparation in the General Guidelines Section of the STR Manual.

2 Digestion

- 2.1 From evidence exam, each nail (or group of nails) should be placed in an irradiated tube.
- 2.2 Add 200 μ L of irradiated 25 mM EDTA/PBS solution to each sample.
- 2.3 Sonicate the samples for one hour at room temperature.
- 2.4 Label a new set of irradiated microcentrifuge tubes with the sample identifiers.
- 2.5 Remove the supernatants from the samples and place in the labeled irradiated microcentrifuge tubes.

3 Extraction

- 3.1 Prepare the digestion buffer according to the calculated volumes. The volumes for one sample are shown below:

Stock Solution	Concentration	1 sample
1.0% SDS	1.0% (0.96%)	2.3 (2.25) μ L
Proteinase K 20 mg/mL	0.80 mg/mL	9 μ L
UltraPure water	N/A	13.7 μ L

- 3.2 Prepare Microcon[®] DNA Fast Flow tubes and label the membrane tube and filtrate tube cap with the sample identifiers. Prepare and label the Microcon[®] collection tubes, sample storage microcentrifuge tubes as well as post-sonication nail collection tubes. The identifier for the post sonication nail collection tubes should include "PS" as a suffix. For example, the post sonication tube for left nail ring finger could be "nail L4 PS".

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- 3.3 **Witness step:** Confirm the sample names on the documentation with the names on all labeled tubes.
- 3.4 Vortex solution well. Add 25 μL of the nail digestion buffer to each sample. Open only one sample tube at a time using the cap opener.
- 3.5 Record the temperatures of the heat shakers. Temperatures must be within $\pm 3^{\circ}\text{C}$ of the set temperature.
- 3.6 Incubate on the heat shaker at 56°C for 30 minutes with shaking at 1400 rpm.
- 3.7 Incubate on the heat shaker at 99°C for 10 minutes with no shaking (0 rpm).
- 3.8 After removing from the shaker, centrifuge the samples at full speed, briefly. Allow the samples to cool for a few minutes while preparing for next steps or chill for 10 minutes at 4°C .
- 3.9 During the digestion period remove the nails using clean tweezers and dry them in a hood. When dry, place the nails in the labeled, post-sonication nail collection tubes.

4 Purification and Concentration

- 4.1 **Self-witness step:** Confirm the sample names on the documentation with the names on the sample and Microcon[®] tubes.
- 4.2 Pre-coat the Microcon[®] membrane with Fish Sperm DNA prepared as follows in an irradiated microcentrifuge tube or 15 mL tube:
 - 4.2.1 Fish Sperm DNA Preparation
 - 4.2.1.1 Add 1 μL of stock Fish Sperm DNA solution (1mg/mL) to 199 μL of water for each sample on the test batch.
 - 4.2.1.2 Aliquot 200 μL of this Fish Sperm DNA solution to each Microcon[®] tube. Avoid touching the membrane. The volume for one sample is shown below. Refer to the extraction documentation for calculated value.

Reagent	1 sample
Water	199 μL
Fish Sperm DNA (1mg/mL)	1 μL

- 4.2.2 NOTE: For samples with 400 μL of digest solution, make a 20 μL solution of 1 μL of Fish Sperm DNA (1mg/mL) with 19 μL of water. Mix well and add this solution to the

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membrane. Ensure that the entirety of the membrane is covered. In this manner, all of the digest may be added to the Microcon[®] membrane for a total volume of 420 μ L.

4.3 Filtration

- 4.3.1 Add the entirety of each extract to its pretreated Microcon[®] membrane. The sample tubes may be discarded.
- 4.3.2 Centrifuge the Microcon[®] tube at 2400 rpm for 12 minutes.
- 4.3.3 Repeat this wash step two more times applying 400 μ L of water onto the membrane and centrifuging again at 2400 rpm for 12 minutes for a total of three washes to remove any residual EDTA.
- 4.3.4 Visually inspect each Microcon[®] membrane tube after the third wash. If it appears that more than 5 μ L remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm.

4.4 Elution

- 4.4.1 Open only one Microcon[®] tube and its fresh collection tube at a time.
- 4.4.2 Add 20 μ L of UltraPure water to the Microcon[®] and invert the Microcon[®] over the new collection tube. Avoid touching the membrane.
- 4.4.3 Centrifuge at 3400 rpm for 3 minutes.
- 4.4.4 Transfer the eluant to an irradiated and labeled 1.5 mL tube. Measure and record the approximate volume. The total volume should not exceed 30 μ L and should not be less than 20 μ L. Adjust the final volume to 20 μ L (if necessary) with UltraPure water. Discard the Microcon[®] membrane.
- 4.4.5 If the eluant appears to be a dark color or is not clear, it may be necessary to purify the sample again. Prepare a fresh Microcon[®] tube and repeat steps 3-4.
- 4.4.6 As needed, pipette aliquots of neat and/or diluted extracts (using TE⁻⁴) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration.
- 4.4.7 Store the extracts at 2 to 8°C or frozen.
- 4.4.8 In LIMS, navigate to the Data Entry page from the Output Samples (extracted DNA), assign the samples to a storage unit (cryobox), and indicate which samples are completed.
- 4.4.9 Have a supervisor review the assay.