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 Manaul.

2 Digestion

2.1 From evidence exam, each nail (or group of nails) should be placed in an irradiated tube.

2.2 Add 200 µL 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.

2.6 Discard tubes containing the actual nails

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) µL
Proteinase K 20 mg/mL	0.80 mg/mL	9 µL
UltraPure water	N/A	13.7 uL

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 and sample storage microcentrifuge tubes.

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 +/- 3°C of the set temperature.

3.6 Incubate on the heat shaker at 56ºC for 30 minutes with shaking at 1400 rpm.

3.7 Incubate on the heat shaker at 99ºC 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

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 199uL 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 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 µL.

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 400uL 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 µL 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 µL 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 µL and should not be less than 20 µL. Adjust the final volume to 20 µL (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.