

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

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

Follow all relevant processes in the [General Guidelines for Forensic Biology and DNA Casework procedure](#).

Follow all relevant processes in the [BEAST DNA Worksheet Setup Manual](#) for creating and adding to worksheets and [BEAST DNA Worksheet Processing Manual](#) for how to record all relevant information while processing the worksheets.

1 Procedure

- 1.1 Turn on heating instruments; Set instruments to 56°C (with shaking), 99°C, and 4 °C. You may need to press Start/Stop to get the Thermomixers started.
- 1.2 Retrieve sample cuttings in 1.5mL tubes. Compare the label on the tubes to the worksheet and confirm you have the correct samples.
- 1.3 Take each sample into your custody.
- 1.4 Obtain two 1.5mL tubes for your extraction negative and label them with the associated extraction negative label.
- 1.5 Retrieve 25 mM EDTA/PBS solution and record the lot number. Add 200µL to each sample and extraction negative.
- 1.6 Place all tubes in the foam tube holder and sonicate the samples for one hour at room temperature.
- 1.7 Print two copies of the tube labels.
 - 1.7.1 Obtain tubes and microcon filters, label them and record the microcon lot number:
 - 1.5mL Eppendorf tubes (using sample label)
 - 1.5mL screw cap elution tubes (using sample label)
 - Microcon tubes and microcon filter (label the tube top and the outside of the microcon filter with a short sample identifier (Example: FN1, RN2 etc.))
 - Three additional sets of microcon tubes (no filter) labeled with the sample identifier for future wash steps.
- 1.8 After the one-hour sonication, briefly centrifuge the samples and transfer the supernatants to the new set of labeled 1.5mL Eppendorf tubes.

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- 1.9 Discard the tubes that contain the actual nails.
- 1.10 Retrieve reagents for the digestion buffer and record the lot numbers. Consult the Mixture Information table for the exact amount of 1.0% SDS, Proteinase K 20 mg/mL, and UltraPure water needed to prepare Digestion buffer::

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

- 1.11 **Tube Setup WITNESS:** Have another analyst verify the tube setup by reading the following for each tube:
- Sample tubes (Sample label)
 - Microcon set-up (Sample identifier written on filter)
 - Final 1.5mL Screw Cap Elution tube (Sample label)

1.11.1 Record the 'Tube Setup Witness'.

- 1.12 Vortex the Digestion Buffer well and add 25 μ L to each sample and extraction negative.
- 1.13 Incubate all samples on a heat shaker set to 56°C (+/- 3°C) for 30 minutes with shaking at 1400 rpm.
- 1.14 Place all samples in the metal screw down rack or use the plastic tube cap locks and incubate on the heat shaker at 99°C (+/- 3°C) for 10 minutes with no shaking (0 rpm).
- 1.15 After removing from the shaker, centrifuge the samples, briefly. Allow the samples to cool for a few minutes while preparing for next steps or chill for 10 minutes at 4°C (+/- 3°C).
- 1.16 Record all instruments and the respective temperature reading on the temperature probes.

2 Purification and Concentration

- 2.1 **Self-WITNESS step:** Confirm the labels on the tubes containing sample and ensure they align in the tube rack with the correctly labeled Microcon tubes.
- 2.2 Retrieve the Fish Sperm DNA and the Water and record the lot numbers.

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- 2.3 In a 15mL conical tube, prepare Fish Sperm DNA by adding 1 μ L of stock Fish Sperm DNA solution (1mg/mL) to 199 μ L of water for each sample on the test batch and vortex. The volume for one sample is shown below.

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

- 2.4 Aliquot 200 μ L of this Fish Sperm DNA solution to each Microcon filter. Avoid touching the membrane.

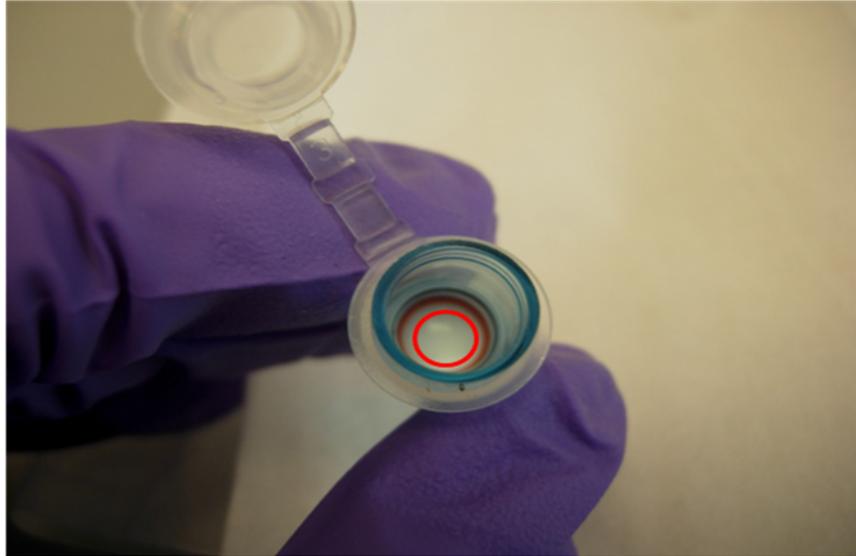
NOTE: For samples with 400 μ L of digest solution, make a 20 μ L solution of 1 μ L of Fish Sperm DNA (1mg/mL) with 19 μ L of water. Mix well and add this solution to the membrane. Ensure that the entirety of the membrane is covered. In this manner, all the digest may be added to the Microcon membrane for a total volume of 420 μ L.

2.5 Filtration

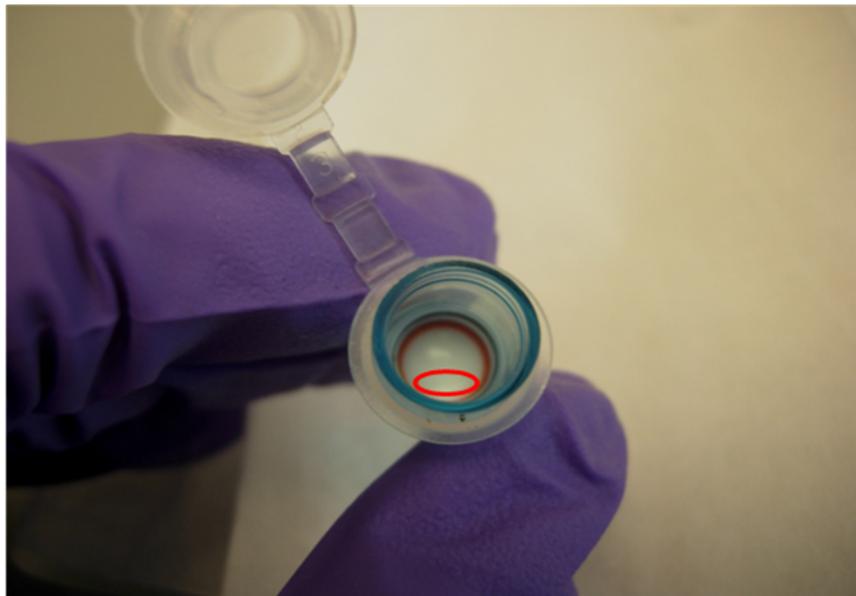
- 2.5.1 Add the entirety of each extract to its pretreated Microcon membrane. The original sample tubes may be discarded.
- 2.5.2 Centrifuge the Microcon tube at 2400 rpm for 12 minutes.
- 2.5.3 Perform a wash step two times applying 400 μ L of water onto the membrane and centrifuge again at 2400 rpm for 12 minutes for a total of three filtration steps to remove any residual EDTA.
- The collection tubes will need to be changed between wash steps to accommodate the full volume for each step.
- 2.5.4 Visually inspect each Microcon membrane tube after the third filtration step. If it appears that more than 5 μ L remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm.
- 2.5.4.1 The Microcon membrane filter should appear barely dry in the center with a faint ring of liquid visible around the edges **before** elution. Please see the images below for clarification:

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The red circle in the above image represents where a faint ring of liquid may be visible around the edges, before eluting.



Alternatively, the red circle in the above image represents where a small area of pooled liquid may be visible, before eluting.

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2.5.5 Lint, bone dust, oils and other particles can clog the membrane. If the filtrate does not appear to be moving through the Microcon membrane, elute the filtrate and continue centrifuging the eluant into a fresh Microcon with a pre-coated membrane. During transfer, pipette off the clear supernatant without disturbing any particle pellet that may have formed. Negative controls should be treated accordingly.

2.5.5.1 In this instance, add a comment in the Run By review task comment section, stating 'Two microcon filters were used for [Sample/Samples] FXX-YYYYY_ sample name, etc. and the associated negative control'.

2.5.5.2 If the problem persists, the specific Microcon lot number might be faulty. Notify the QA Unit and try a different lot number.

2.6 Elution

2.6.1 Add 20 μ L of UltraPure water to the Microcon and invert the Microcon over the new collection tube. Avoid touching the membrane.

2.6.2 Centrifuge at 3400 rpm for 3 minutes.

2.6.3 Transfer the eluant to the 1.5mL screw cap elution tube and measure and record the volume to the nearest tenth of a microliter in the 'Volume DNA' column.

- The total volume should not exceed 30 μ L and should not be less than 20 μ L.
- Adjust the final volume to 25 μ L (if below 25 μ L) with UltraPure water and record the volume of water used in the 'Volume H₂O' column. Discard the Microcon membrane.

2.6.4 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 step 2.5.

2.7 Record the 'Extraction Run By' review task and 'Response' dropdown for the 'Pass or Fail?' step.

2.8 Transfer custody of all extracts to a cryobox and store in a refrigerator or freezer.

2.9 Assign samples to next process step.