

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

HIGH YIELD DNA EXTRACTION		
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High Yield DNA Extraction

A. Preparation

1. Extraction sets consist of 9 samples and one or two extraction negatives. Additional extractions may continue sequentially during incubations.
2. Name the extraction set by its date and time using the following format: "082010.1000". An "E" may precede the date and time of the extraction.
3. The documentation will automatically calculate the requisite amount of reagents needed for the extraction.
4. **Follow the procedures for Work Place Preparation (refer to the General Guidelines Procedure of this manual).**

B. Digestion

1. **Self-Witnessing Step:** Confirm the sample names and their order on the documentation with the names on the sample tubes.
2. Obtain reagents and record lot numbers.
3. Prepare digestion buffer in an UV irradiated tube (1.5 mL, 2.0 mL Dolphin, or 15 mL).
4. Prepare the digestion buffer according to the calculated volumes on the documentation. The volume for one sample is shown below.

Stock Solution	Concentration	1 sample
0.05% SDS (or 0.01% SDS when using Poly A RNA at a later step)	0.05% (or 0.01%)	192 µL
Proteinase K 20 mg/mL	0.80 mg/mL	8 µL

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5. Vortex solution well. Add **200 µL** of the digestion buffer to each sample. Open only one sample tube at a time using the cap opener. Ensure that the swabs are submerged in the digestion fluid. If necessary, add an additional 200 µL of the digest buffer (including the Proteinase K) to the sample in order to submerge a large sample, and be sure to document the deviation.
6. Record the temperatures of the heat shakers. Temperatures must be within $\pm 3^{\circ}\text{C}$ of the set temperature.
7. Incubate on the heat shaker at 56°C for 30 minutes with shaking at 1400 rpm.
8. Incubate on the heat shaker at 99°C for 10 minutes with no shaking (0 rpm).
9. Place sample in cold block at 4°C for 10 minutes with no shaking (0 rpm).
10. Centrifuge the samples at full speed, briefly.
11. During the digestion period label the Microcon[®] DNA Fast Flow and elution tubes, and print labels for storage tubes.

C. Purification and Concentration

1. Prepare Microcon[®] DNA Fast Flow tubes and label the membrane tube and filtrate tube cap.
2. **Witness step:** Confirm the sample names and order on the documentation with the names on the sample and Microcon[®] tubes.
3. Pre-coat the Microcon[®] membrane with Fish Sperm DNA in an irradiated microcentrifuge tube or 15 mL tube:
 - a. Fish Sperm DNA Preparation
 - i. Add 1 µL of stock Fish Sperm DNA solution (1mg/mL) to 199µL of water for each sample on the test batch.

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- ii. 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 documentation for calculated value.

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

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. Filtration

- a. Add the entirety of each extract to its pretreated Microcon[®] membrane. If this is a purification/concentration assay of a sample that has already been extracted and the sample volume is lower than 200 μL , raise the sample volume to 200 μL with dH₂O. Aspirate all of the solution from the sample tube by placing the pipette within the swab. The sample tubes may be discarded.

Centrifuge the Microcon[®] tube at 2400 rpm for 12 minutes. An additional 3 minutes may be required to ensure that all the liquid is filtered. However, do not centrifuge too long such that the membrane is dry. If the filtrate does not appear to be moving through the membrane, elute the filtrate and continue centrifuging the eluant into a fresh microcon with a pretreated membrane.

If indicated on the evidence examination schedule or by a supervisor, or if the filtrate is not clear, perform a second wash step applying 400 μL of water onto the membrane and centrifuging again at 2400 rpm for 12 minutes or until the all the liquid is filtered. However, do not centrifuge to dryness. This process may be repeated, as necessary. Document the additional washes.

All samples undergoing extraction with 0.05% SDS must be purified and concentrated a second time by repeating this section (Section C).

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- b. Visually inspect each Microcon[®] membrane tube. If it appears that more than 5 μL remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm.

5. Elution

- a. Open only one Microcon[®] tube and its fresh collection tube at a time.
- b. Add 20 μL 0.1X TE to the Microcon[®] and invert the Microcon[®] over the new collection tube. Avoid touching the membrane.
- c. Centrifuge at 3400 rpm for 3 minutes.
- d. 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 using 0.1X TE (if less). Discard the Microcon[®] membrane.
- e. 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 4-5.
- f. Store the extracts at 2 to 8°C or frozen.
- g. In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and indicate which samples are completed.
- h. Have a supervisor review the assay.