FBS10- Microcon Procedure Microconcentration of DNA Samples

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1. Scope

1.1. This procedure is used to concentrate and/or purify the DNA extracted from a sample.

2. Background

2.1. To establish the practices for documenting the examination of evidence to conform to the requirements of the Department of Forensic Sciences (DFS) Forensic Science Laboratory (FSL) *Quality Assurance Manual*, the accreditation standards under ISO/IEC 17025:2005, and any supplemental standards.

2.2. Although microconcentration is incorporated into the organic extraction method(s), it can also be utilized independently as a supplemental technique for concentrating a high volume sample, eliminating possible inhibitors following quantitation or eliminating primers post-amplification. The Millipore Microcon DNA Fast Flow uses a low-binding, anisotrophoic, hydrophilic regenerated cellulose membrane to filter out unwanted particles contained within the sample which are below the size of 100,000 Daltons or 300 single stranded DNA nucleotides (150 double stranded DNA nucleotides). The contents of the filter can then be recovered and reconstituted, generating a cleaner and/or more concentrated sample.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.

3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

4.1. Microcon DNA Fast Flow centrifugal filter devices
4.2. Microcon tubes
4.3. TE Buffer
4.3.1. NOTE: Never use solutions directly from the stock bottles. Use Reagent SOPs for preparation and labeling instructions.

5. Standards and Controls

5.1. An additional negative control (RB-TE) is used if the procedure is being used post-amplification OR if the TE Buffer used during extraction is no longer available for use during microconcentration. If multiple samples are being microcon'd for both cleanup and concentration, a novel RB-TE must be prepared for each microcon process.

6. Calibration

6.1. Not applicable

7. Procedures

7.1. Vortex and spin down all the samples to be concentrated, including controls.

7.2. Obtain and label the upper reservoir of the assembled Microcon DNA Fast Flow unit with the case and sample numbers.

7.3. Add the DNA sample to the upper reservoir, being cautious to avoid touching the filter with the pipette tip. The sample may be added alone or with additional TE Buffer to a maximum volume of 500µl. Record the total volume of the sample added to the reservoir. Record the DNA concentration (quant value), if known.

7.4. Place the samples in a microcentrifuge for 10-25 minutes at 500g. Alternatively, the samples can be centrifuged until the fluid is completely drawn through the filter.

7.5. Label a new set of Microcon tubes with the case number and sample name.

7.6. When the fluid has been drawn completely through the filter, add 25 ul of TE buffer to all reservoirs, and invert the upper reservoirs into the recovery tubes. Microcentrifuge for 3 minutes 500g. Be certain that the caps on the tubes are all facing inward in the microcentrifuge to avoid possible snapping of the caps.

7.7. Remove and discard the upper reservoir columns from each tube. Measure the amount of sample in the new tubes and record this volume as "volume sample recovered."

7.7.1. **NOTE:** If the microcon procedure was performed to clean the DNA extract, proceed to step 8. If the microcon was performed to concentrate the DNA, skip step 8 and proceed to step 9.

7.7.2. **IMPORTANT:** All negative controls (RB, RRB, RB-TE, etc.) must be equal to the lowest volume of any sample for either the cleanup or concentration step.

7.8. For sample cleanup- add TE buffer to the recovered sample to equal the initial volume of the sample recorded in step 3.

7.9. For sample concentration- add TE buffer, if necessary, to bring the final sample volume to 25ul.

7.10. The new DNA concentration (C2) can be calculated based on the initial DNA concentration (C1), (if known), the initial volume (V1), and the final volume (V2) using the following formula:

$$C2 = (C1 \times V1) / V2$$

7.11. If the initial quant value is unknown, or undetermined by the first quantitation, then submit an aliquot of the microcon'd DNA, along with an aliquot of the negative control, to quantitation.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. The new DNA concentration (C2) can be calculated based on the initial DNA concentration (C1), (if known), the initial volume (V1), and the final volume (V2) using the following formula:

$$C2 = (C1 \times V1) / V2$$

10. Uncertainty of Measurement

10.1. When quantitative results are obtained, and the significance of the value may impact the report, the uncertainty of measurement must be determined. The method used to determine the estimation of uncertainty can be found in the *FSL Quality Assurance Manual – Estimation of Uncertainty of Measurement (Section 5.4.6).*

11. Limitations

11.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.

11.2. Caution should be taken to prevent microcon filters from exceeding their specified limitations. Excessive g-force may result in leakage or damage to the centrifugal device.

12. Documentation

12.1. FBU Microconcentration Worksheets

13. References

13.1. Microcon ® Centrifugal Filter Devices – User Guide

13.2. *Forensic Science Laboratory Quality Assurance Manual* (Current Version)

13.3. *FSL Departmental Operations Manuals* (Current Versions)

13.4. *FSL Laboratory Operations Manuals* (Current Versions)

13.5. *FBS08 - Organic DNA Extraction* (Current Version)

13.6. *FBS09 – Differential Organic DNA Extraction* (Current Version)