Microcon DNA Fast Flow DNA Concentration and Purification		
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Microcon DNA Fast Flow DNA Concentration and Purification

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 Overview

1.1 Microconning a DNA extract is useful when attempting to concentrate it, clean it of lysate and chemical inhibitors or both. The procedure differs slightly depending on which of these results are desired.

1.2 Microconning can also be used to combine duplicate DNA extracts (for example, when there is a _A and _B replicate from an Extraction that needs to be recombined). Combination microcons can be performed on any Microcon type, although a new quantitation should be performed to obtain the most accurate value.

1.3 'Microcon to concentrate' – bringing the total volume of the DNA extract down, therefore concentrating the DNA; initial and final volumes are recorded, and the new concentration is calculated by $C_1V_1 = C_2V_2$ in the LIMS Data Entry.

1.4 'Microcon to clean' – when cleaning or purifying a DNA extract, it is necessary to perform a wash step with a solution (i.e., TE^{-4} or water); the initial volume is recorded, and the elution is returned to that same volume. The concentration of the DNA extract remains the same.

1.5 'Microcon to clean and concentrate' – a combination of both steps; the wash step is performed, and the total volume of the DNA extract is brought down. A new quantitation should be performed to obtain the most accurate value, although the new concentration may be calculated in the LIMS Data Entry.

1.6 To allow for duplicate amplifications, the final volume should be at least 25µL. If the starting volume is <25µL notify the lab supervisor or your supervisor before proceeding.

2 Assay Preparation

2.1 Retrieve samples needed for microcon and take each sample into your custody.

2.2 Obtain a 1.5mL tube for your microcon negative and label it with the associated microcon negative label.

2.3 Print two copies of the tube labels.

2.3.1 Obtain tubes and microcon filters, label them and record the microcon lot number.

- Microcon tube (sample label)
- Microcon filter (label the outside of the microcon filter with a short sample identifier (Example: FN1, RN2 etc.))
- Final 1.5mL Screw Cap Elution tube (Sample label)

2.4 Select which Microcon process (Clean, Concentrate, Clean & Concentrate) is being performed from the *Processed* dropdown.

2.5 Retrieve reagents and record the lot numbers. Consult the Mixture Information table for the exact amount of UltraPure Water and Fish Sperm DNA (1mg/mL) needed to prepare the master mix.

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

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

2.6 Aliquot 200µL of this Fish Sperm DNA solution to each Microcon filter. Avoid touching the membrane.

2.7 Process 50µL of TE^{-4} solution as a Microcon negative control. Make sure to use the same lot that will be used to dilute the samples.

2.8 Briefly centrifuge each sample.

2.9 **Tube Setup WITNESS**: Have another analyst verify the tube setup by reading the labels on each tube.

2.9.1 Record the 'Tube Setup Witness'.

2.10 Record the initial sample concentration for each sample in the 'Starting Conc.' column.

2.11 Measure the initial sample volume for each sample using an adjustable Micropipette, to the nearest tenth of a microliter, and record the volume in the 'Initial Volume' column.

2.11.1 For a sample being combined/pooled, select either of the current sample concentrations for the "Starting Conc.' and the combined volume for the 'Initial Volume'. The resulting sample should be re-quantified to obtain the most accurate concentration.

2.12 Add each sample (400µL maximum volume) to the buffer in the microcon filter. Don't transfer, any organic solvent. Seal with attached cap. Avoid touching the membrane with the pipette tip.

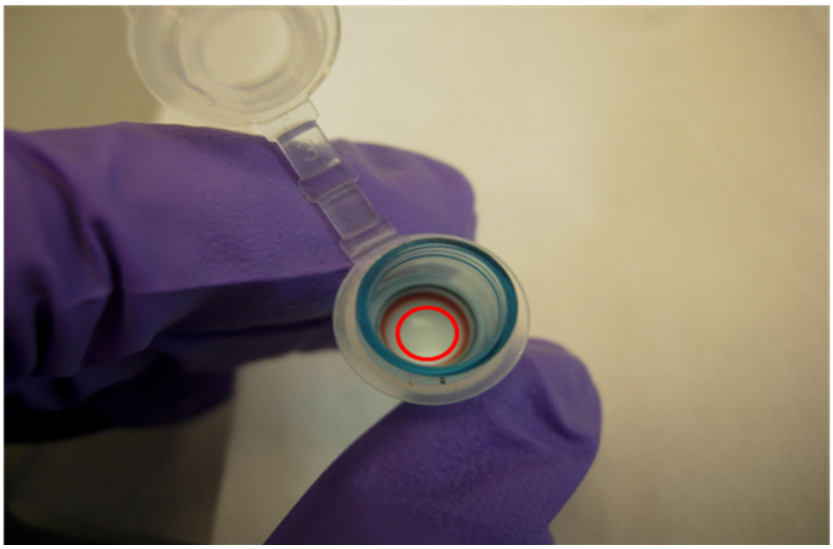
2.13 Return the extraction tubes to their original storage location. Do not discard the empty tubes.

2.14 Place the Microcon assembly into a centrifuge and ensure the tubes are properly balanced.

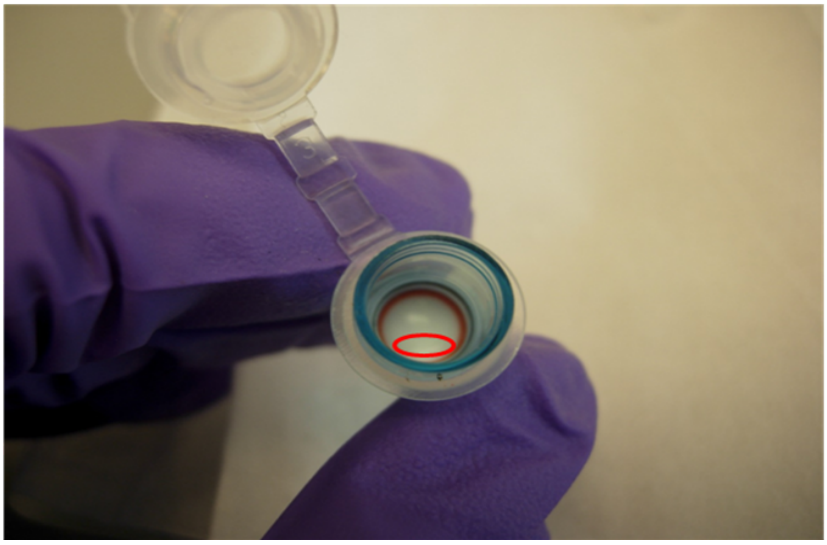
2.15 Centrifuge at 2400 RPM for 12 minutes. To prevent failure of the microcon filter, do not exceed recommended speed. Additionally, do not centrifuge too long (the membrane should not be allowed to become completely dry).

2.16 After the initial 12 minutes, visually inspect each Microcon tube. If it appears that more than 2µL remains above the membrane, centrifuge that tube for 3 more minutes at 2400 rpm. This process may be repeated as necessary. Do not centrifuge too long (the membrane should not be allowed to become completely dry).

2.16.1 The Microcon membrane filter should appear barely dry in the center with a faint ring of liquid visible around the edges before purification or elution. Please see the images below for clarification:



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.

2.17 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.

2.17.1 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."

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

2.18 ***PURIFICATION – ONLY perform for 'Microcon to clean' or 'Microcon to clean and concentrate' Microcon assays***(otherwise, skip to Step 2.19):

2.18.1 Transfer the filter to a new collection tube, then add 200µL of TE^{-4} solution to the Microcon membrane, carefully pipetting up and down in order to re-suspend the DNA into solution and repeat Steps 2.144. – 2.16.

2.18.2 Do this as often as necessary to generate a clear extract and then continue with to Step 2.19. When performing multiple wash steps, it may be necessary to empty the bottom collection tube intermittently.

2.18.3 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 above for clarification.

NOTE: When purifying samples with a low DNA concentration it may be advantageous to perform a **'Microcon to clean and concentrate' assay** with several wash steps and to also reduce the volume; this leads to both a cleaner sample and an increased DNA concentration.

2.19 Once the sample is ready to elute, add 20µL TE^{-4} to the sample reservoir Avoid touching the membrane with the pipette tip.

2.20 Separate the collection tube from the sample reservoir and place the microcon filter upside down in a new labelled collection tube.

2.21 Centrifuge at 3400 RPM for 3 minutes. Ensure sure all tubes are balanced.

2.22 Transfer the eluant to the 1.5mL screw cap tube and measure and record the volume to the nearest tenth of a microliter; adjust volume to desired level using TE^{-4}. Record the resulting volumes and volume adjustments in the final elution volume in the 'Final Volume' column for each sample.

2.22.1 '**Microcon to concentrate' assay:** low DNA concentration samples sent for microcon concentration are to be reconstituted to a minimum of 25µL and a maximum of the initial volume.

2.22.2 **'Microcon to clean' assay:** high DNA concentration samples sent for microcon clean-up are to be reconstituted to their initial volume.

2.22.3 **'Microcon to clean and concentrate' assay:** DNA samples sent for microcon clean-up and concentration are to be reconstituted to a minimum of 25µL and a maximum of the initial volume.

2.23 Ensure that the new concentration is correctly calculated for each sample in the 'Resulting Conc.' column.

NOTE: The initial and calculated concentrations for samples that have not yet been quantified will be listed as '0pg/µL'.

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

2.25 Transfer custody of all samples to a cryobox and store in a refrigerator or freezer.

2.26 Assign samples to next process step.

ATTENTION: DO NOT store the DNA in the Microcon vials. The lids are not tight enough to prevent evaporation.