Forensic Biology Section

Centricon DNA Filtration

1. Scope

1.1. The Centricon-100 centrifugal filter device concentrates solutions of DNA by ultrafiltration in a fixed-angle rotor centrifuge.

1.2. The devices are used to filter or concentrate in the following instances:

1.2.1. When very low yields of DNA are obtained after the extraction procedure, the volume of DNA extract can be reduced in order to increase the extract's concentration so more DNA can be added to the amplification reaction.

1.2.2. When the presence of PCR inhibitors are suspected, the DNA extract can be filtered and then brought back up to a suitable volume for amplification in an attempt to remove the inhibitors.

1.2.3. As the final clean-up step following phenol/chloroform organic extractions.

2. Safety

2.1. WARNING: CHEMICAL HAZARD. Wear chemical-resistant gloves and eye protection when handling phenol-chloroform solution. Use in well-ventilated area.

2.2. Treat all biological specimens as potentially infectious. Gloves and a laboratory coat must be worn at all times. Follow Universal Precautions.

3. Specimen

3.1. Phenol/chloroform DNA extracts.

3.2. DNA extracts containing a low concentration of DNA.

3.3. DNA extracts believed to contain PCR inhibitors.

4. Equipment

4.1. Fixed-angle centrifuge (e.g. Eppendorf 5415 or 5430)

5. Procedural Note

5.1. Do not autoclave or irradiate the Centricon filter device.

5.2. Avoid touching the Centricon membrane with the pipette tip.

5.3. Avoid transferring any phenol/chloroform organic phase to the filter device.

6. Assembling the device

6.1. Assemble and label a Centricon device for each sample to be filtered (including Reagent Blanks) by inserting a "sample reservoir" into a "filtrate vial".

6.2. Place a "retentate vial" on the top of the "sample reservoir" to close the assembly, and label the "retentate vial" with case number and item number.

6.3. Also label an extra microcentrifuge tube for each sample (for final storage).

7. Filtering DNA extracts

7.1. Carefully transfer the entire DNA extract to the "sample reservoir" (upper chamber) of the Centricon (up to 2 ml of aqueous phase can be transferred at once).

7.2. Centrifuge for at least 15 minutes at **500 x g** (2136 rpm in Eppendorf 5430; 2400 rpm in Eppendorf 5415). Repeat centrifugation step until almost all liquid is filtered through the membrane, leaving only a residue of liquid at the edge

7.3. NOTE: If liquid is slow to pass through the filter, the following may have occurred:

7.3.1. The filter may be clogged (in which case, the retentate should be transferred to a new filter device).

7.3.2. The DNA solution may be too concentrated (in which case, the sample may be quantitated at whatever volume has been achieved).

7.3.3. The sample may be too cold to pass through the filter efficiently (in which case, the sample needs to warm up to room temperature).

7.4. If filtering an organic extraction: add 2 ml of TE^{-4} buffer to the "sample reservoir" and repeat the centrifugation in step 7.2 above.

7.5. Turn "retentate vial" and "sample reservoir" upside down in the "filtrate vial." Spin for 3 minutes at **1000 x g** (3021 rpm in Eppendorf 5430; 3500 rpm in Eppendorf 5415), or pulse briefly, to transfer retentate from filter into the "retentate vial."

7.6. Transfer retentate to a labeled microcentrifuge tube for final storage.

7.7. Bring retentate up to volume with TE^{-4} buffer if necessary, for a final volume of as little as 20 µl, or as much as 200 µl. The sample is now ready for quantitation and the PCR amplification process.

7.8. The sample can now be stored in a refrigerator (1° C - 8° C) for short-term storage, stored in a freezer (-10° C or colder) for long-term storage, or processed in downstream applications.