1. Scope

This protocol is used to concentrate and/or further purify DNA extracts. DNA typing will not be successful if there is insufficient starting material during the amplification process. Additionally, the presence of certain substances called inhibitors (e.g. fabric dyes) may prevent amplification from occurring. Microcon® filters offer a means of concentrating DNA and removing the inhibitors from a DNA extract. During centrifugation, the filter captures larger molecules (DNA), and allows the liquid and smaller molecules (inhibitors) to pass through. In the final step, the DNA is eluted from the filter in a volume of liquid determined by the analyst. This method may be used to concentrate DNA extracts down to as little as 10 µL, if desired. While the Microcon® can be used to further purify a DNA extract, it may not remove all inhibitory substances. A small percentage of the DNA in the original extract may be lost during the Microcon® filtration. It is important to weigh the benefit of concentrating and/or further purifying the DNA with the possibility of losing a portion of the DNA.

2. References

2.1. Millipore Microcon® Centrifugal Filter Devices User Guide, June 2005.
2.2. Internal validation studies.
2.3. C.T. Comey, B.W. Koons, K.W. Presely, J.B. Smerick, C.A. Sobieralski, D.M. Stanley, F.S. Baechtel, DNA Extraction Strategies for Amplified Fragment Length Polymorphism Analysis, J. Forensic Sci. 39 (1994) 5.
2.4. A.M. Krowczynska, K. Donoghue, L. Hughes, Recovery of DNA, RNA, and protein from gels with Microconcentrators, Biotechniques, 1995, 18(4): pp. 698-703.

3. Equipment

3.1. Disposable gloves
3.2. Eye protection
3.3. Lab coat
3.4. 30 kD Microcon® (sample reservoir and retentate/filtrate vials)
3.5. TE^{-4} (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)
3.6. Carrier RNA (cRNA, 1 µg/µl, Qiagen)
3.7. 10% bleach solution
3.8. 70% EtOH
3.9. Vortexer
3.10. Pipettes
3.11. Benchtop hood
3.12. Disposable aerosol-resistant pipette tips
3.13. Centrifuge

4. Safety/Quality Assurance

4.1. Disposable gloves shall be worn during this procedure.
4.2. Perform concentration steps, where possible, in a hood to reduce the risk of contamination. Clean surfaces with 10% bleach solution prior to use. After exiting the hood, turn on the UV light.
4.3. Use aerosol-resistant pipette tips when transferring liquids containing DNA.
4.4. Change pipette tips after transferring any liquids potentially containing DNA.
4.5. Record the lot number of each reagent used in notes. Do not use the reagents after the expiration date.
4.6. The reagent blank must be concentrated with associated DNA extracts.
4.7. Lab coat and eye protection must be worn at all times while performing this procedure.
4.8. Where practical, only tubes associated with one sample shall be open at a time.
4.9. Final DNA extract tubes shall be labeled, at a minimum, with case number, item number, and analyst's initials.
4.10. In general, samples thought to contain significantly lower levels of DNA will be handled before those thought to contain large amounts of DNA.
4.11. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Procedure

5.1. In benchtop hood, appropriately label the assembled filters (sample reservoir and retentate/filtrate vial) and extra vials. The clear side of the sample reservoir should be facing upwards.
5.2. Add 1 µl of cRNA (1 µg/µl) to each DNA extract tube being concentrated.

NOTE: If combining samples, cRNA should only be added to one of the extracts being combined for concentration.

5.3. Vortex and briefly centrifuge samples to remove extract from the inside of lid.
5.4. Transfer the DNA extract into the sample reservoir without touching the membrane with the pipette tip.

NOTE: **Multiple extracts can be combined at this step if desired. Up to 500 µl of volume can be added to the sample reservoir at a time.**
NOTE: If combining multiple extracts, an equal number of reagent blanks that were extracted concurrently with the evidentiary samples shall be combined and concentrated to the same level as the evidentiary sample(s) with the lowest volume. The reagent blanks to be combined should include any reagent blanks with a detectable DNA concentration as determined during quantitation. If no reagent blanks exhibit a detectable DNA concentration, then the reagent blanks will be selected in order 1 through X (X being the number of evidentiary samples combined). If multiple DNA extracts from the same exhibit are combined, the combined DNA extract sub-item may be designated as X.Q combo (where "X" refers to the original exhibit number). If DNA extracts from multiple exhibits are combined, the combined DNA extract sub-item shall reflect all of the original exhibits. For example, if DNA extracts from two pieces of an end cap (Exhibits 1 and 2) are combined, the combined sub-item may be designated as Exhibit 1-2.Q combo. If no DNA extracts are combined and more

than one reagent blank was initiated, all reagent blanks that demonstrated a detectable DNA concentration, as determined during quantification, shall be processed to completion. If no reagent blanks demonstrated a detectable DNA concentration as determined during quantification, the first reagent blank of the set shall be processed to completion. The unused reagent blanks shall be retained, unless all of the associated evidentiary DNA extracts are consumed in analysis.

5.5. Close the filter assembly and place in the centrifuge. Spin at 14,000 x g for approximately 4-6 minutes. Spin time will vary based on starting volume. Do not excessively dry the membrane.

5.6. Inspect bottom of sample reservoir. If excess liquid is present, filter assembly may be spun for additional time.

5.7. Add 100 µl of TE^{-4} to the reservoir for each sample and spin at 14,000 x g for approximately 4-6 minutes. Spin time may vary. Do not excessively dry the membrane.

Optional: Additional washes with TE^{-4} buffer may be performed to further purify DNA extract. Pipette desired wash amount (up to 300 µL) into the sample reservoir and repeat step 5.5. If performing repeated washes, additional vials may be required.

5.8. Remove sample reservoir from vial containing filtrate and invert sample reservoir (white side up) into a new labeled vial.

Optional: Pipette additional TE^{-4} buffer (1-40 µL) into bottom of sample reservoir prior to inversion. Reservoirs may be gently agitated using low setting on vortexer after inversion. Discard vial containing filtrate.

5.9. Spin at 1,000 x g for 3-5 minutes to collect the concentrated DNA extract in the new vial.

Optional: After the recovery spin, additional TE^{-4} buffer may be added to DNA extract to achieve desired volume.

5.10. DNA extracts can be stored at 4ºC for short periods and should be stored at -20ºC for longer term storage.

NOTE: If the DNA extract(s) is/are stored at -20°C for a longer period of time, the extract may be transferred to a properly labeled 1.5 ml or 2 ml microcentrifuge tube.