DNA EXTRACT CONCENTRATION AND/OR PURIFICATION

Background

This procedure allows for the concentration and/or purification of DNA extracts using micro-concentrator units.

Summary of Procedure

Micro-concentrator units are used to purify samples that possibly contain PCR inhibitors and / or to concentrate samples that contain an unacceptably low amount of DNA. The procedure can be used on quantitated or un-quantitated DNA extracts. The micro-concentrator unit contains a membrane that binds all DNA but allows the original elution buffer and possible inhibitors to pass through it, into a waste vial. If the sample requires concentration, then the filter unit is merely turned upside down into a clean sample tube and the DNA is removed from the membrane in a new volume of approximately 20-40uL. If the sample only requires purification and not concentration, MBG water is added to the unit just before it is inverted and spun – eluting the sample in the same volume (or even a greater volume if desired) as it was originally eluted in.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**

Reagents and Materials

10% Bleach
Bench Paper
Microcon Centrifugal Filter Devices and collection tubes
Micro-Centrifuge
Vortex
Single Channel Pipettes (40uL and 200uL)
Filtered Tips, sterile
Gloves
Lab Coat
KimWipes
MBG Water

Reagents and Materials – Storage and Handling

All reagents and materials are kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **DNA Concentration Worksheet** is required for documentation. All information must be completed.

Procedure

1. Label two clean collection tubes (that come with the micro-concentrator units) for each sample to be concentrated or purified.

   A witness must check the setup of the concentration tubes and extracts against the worksheet and initial the appropriate space on the worksheet.

2. Prepare volume standards for use in step 11 by adding several different volumes to empty collection tubes. Label the tubes according the volume contained within. There should be at least three standards prepared for each desired volume. For example, if the desired volume for a sample is 40uL then a 20uL, a 40uL and a 60uL standard should be prepared.

3. Place a clean blue filter unit into one of the labeled collection tubes for each sample.

4. If known, record the Original Extract Concentration on the DNA Concentration Worksheet.

5. Pre-wet the microcon filter with at least 5uL.

6. Add the entire volume of the DNA extracts into the blue filter units and record the volume (Original Extract Volume) on the DNA Concentration Worksheet.

7. Cap the tubes and centrifuge at 2000rpm for 6 minutes. This step allows most of the elution buffer to pass through the filter unit while the DNA remains bound to the filter membrane. Sample volume should be reduced but the membrane should not appear dry. Additional centrifugation at 3000rpm may be needed for some samples.

8. If concentration of the sample is desired, remove the blue filter unit from the collection vial and place it upside down into the second collection vial labeled for that sample. Discard the first collection vial containing the original elution buffer into a biohazard waste container.

   If the sample only requires purification, add the desired volume of MBG water to the blue filter unit prior to inverting it in the second tube.

9. Centrifuge the inverted micro-concentrator unit for 3 minutes at 3000 rpm. This step will release the DNA in an adjusted volume into the clean collection vial.

   Note: If the volume is lower than desired, remove the blue filter unit from the collection vial, add the desired volume of molecular biology grade water to the filter membrane, invert and insert the blue filter unit (inverted) back into the collection vial and repeat step 9.

   If the volume is higher than desired, add the recovered volume back into the blue filter unit (right side up) and repeat step 6.

10. Once the desired volume is achieved, discard the blue filter unit into a biohazard waste container. The DNA extract will be permanently stored in the final collection tube.

11. Estimate the New DNA Volume by visually comparing the amount of liquid in the tube to the previously prepared volume standards and record on the DNA Concentration Worksheet.

12. If the concentration of the extract was known before concentration, calculate the New DNA Concentration in each sample and record on the DNA Concentration Worksheet:

    New DNA Concentration (ng/uL) = Original Extract Concentration (ng/uL) X Original Extract Volume (uL)

New DNA Volume (uL)

If the extract was not quantitated prior to this procedure, quantitate now. Use the above formula to calculate the Original Extract Concentration and record on the DNA Concentration Worksheet.

13. The extract is now ready for amplification set-up.

14. Decontaminate all work areas with appropriate solutions.

Comments on Storage

Non-amplified DNA must be stored separately from amplified product. Store samples at 2-8°C.

Technical Assistance

For information and assistance, contact Millipore at 1-800-645-5476.

Reference

Microcon® Centrifugal Filter Devices User Guide Rev. J, 03/00