DNA EXTRACT DILUTION

Background

This procedure allows for the dilution of DNA extracts for optimal amplification.

Summary of Procedure

Molecular biology grade water may be used to dilute extracts that contain a high concentration of DNA prior to amplification. Samples must be quantitated prior to dilution. The same preparation of MBG water used to amplify the sample should be used to dilute the extract, so that the amplification negative control can also be used as a reagent control for the dilution procedure.

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
Micro-centrifuge tubes
Micro-centrifuge
Vortex
Single Channel Pipettes (40uL and 200uL)
Filtered Tips, sterile
Gloves
Lab Coat
KimWipes
MBG Water
TE^{-4}

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 Dilution Worksheet** is required for documentation. All information must be completed.

Procedure

1. Allow the DNA extracts to equilibrate to room temperature.
2. Quantitate the extracts if not already done so.

3. Record the extract concentration of each sample to be diluted on the DNA Extract Dilution Worksheet.
4. Determine what the end concentration of each sample should be and then determine a dilution factor that will allow for that concentration. Dilution factors that are multiples of 2 or 10 are easiest to calculate. The following formula should be used:

   Dilution Factor (DF) = Original Concentration (C1) / Desired Concentration (C2)

5. Label one clean 1.7mL micro-centrifuge tube for each sample to be diluted.

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

6. Vortex the extracts to be diluted thoroughly and spin down briefly in a micro-centrifuge.
7. Add 5uL of the samples to the appropriate volume of MBG water or TE^{-4} to achieve the desired final concentration.

   For example: If the original concentration of a sample is 16ng/uL, a dilution factor of 100 will result in an end concentration of 0.16ng/uL. If 5uL of the sample is added to the dilution tube, then 495uL MBG water should be added to achieve the 1:100 dilution.

8. The diluted extract is now ready for amplification set-up.
9. 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.