

DNA Analyst Training Laboratory Training Manual

Protocol 5.01 Calculations and Dilutions Procedure



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the National Forensic Science Technology Center. It has been included for training and example purposes only.

**PRESIDENT'S
DNA
INITIATIVE**



INTRODUCTION

Amplification of an appropriate input quantity of DNA is important to optimize STR results for a sample. From the quantitation results, the concentration of each sample is estimated. According to the concentration calculated for a sample, the extract is amplified as is, or diluted to achieve the desired input quantity of DNA for amplification.

SAFETY CONSIDERATIONS

Refer to the Laboratory Safety Manual(s)

PREPARATIONS

None

INSTRUMENTATION

None

MINIMUM STANDARDS & CONTROLS

None

PROCEDURE OR ANALYSIS

Calculations

1. Quantitation results (ng) of sample/ μl of sample quantitated = ng/ μl
2. Concentration/desired concentration for amplification* = dilution factor

*The volume of the sample contributing to the total amplification reaction volume must be considered; i.e. if 10 μl of sample volume is required with a 1 ng total quantity, the DNA is to be diluted to 0.1 ng/ μl .

Example

A sample produces a result of 5 ng of DNA and the quantitation protocol requires the use of two μl of DNA extract, the use of formula (1) results in a result of 2.5ng/ μl for that sample.

If 1.25 ng is the optional amount of template DNA to use with the STR multiplex and the multiplex reaction requires 10 μl of an extract, formula (2) is used to determine the dilution. Thus, one could take 2 μl of the stock extract, mix it with 39 μl sterile H₂O and add 10 μl of this dilution to the amplification mixture.

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