

VALIDATION OF Philisa® Streck THERMAL CYCLER FOR FORENSIC DNA ANALYSIS OF CHEMICALLY FIXED TISSUE

Jessica Bouchet, The Pennsylvania State University

An integral aspect of analyzing biological evidence retrieved from crime scenes is the use of polymerase chain reaction (PCR) DNA technology, which allows an analyst to obtain short tandem repeat (STR) DNA profiles from minute amounts of body fluids. The instruments and methods currently used and employed in forensic laboratories for the thermal cycling process typically take up to three or more hours. In this research, the thermal cycling time was successfully reduced to an average of 17 minutes using a new innovative instrument.

Tissue samples were obtained from three deceased individuals (two female and one male). Five tissue types were collected from each of the donors including small bowel, kidney, colon, liver and muscle. Gall bladder was also collected from one individual. These samples were fixed in 100% alcohol for varying length of time, which ranged from 4 weeks to 24 weeks. The chemically fixed tissues as well as the pristine, non-preserved samples were stored in the freezer until needed for analysis. Every donor and each sample used in this study was anonymized as required by The Pennsylvania State University.

The extracted DNA from fixed and unfixed tissue samples were quantified using a Qubit® 3.0 Fluorometer, which provided the quantity of DNA in each extracted sample (ng/µL). With this information, the necessary volume of the extract needed for amplification was determined. The target was to add 1.0 ng of DNA in the reaction mixture as recommended by the manufacturer of the amplification kit, the PowerPlex® Fusion System. This kit amplifies the alleles in 24 loci as part of the research project. The samples were then prepared for amplification using the Philisa® Streck Thermal Cycler. After amplification of fixed and unfixed samples, DNA fragments were analyzed using a 3130xL Genetic Analyzer to evaluate the success of the amplification and the concordance of the profiles. The data files retrieved from the 3130xl were analyzed using GeneMarker HID 2.7.1 from SoftGenetics. The profiles generated were compared to the known profiles obtained from unfixed tissues of the corresponding donor. DNA profiles were successfully obtained from the fixed and unfixed tissues, amplified with the PowerPlex® Fusion System using the Philisa® Streck thermal cycler. The data were concordant with published data previously generated from the same fixed tissues using the 9700 thermal cycler. The amplification was completed in 17 minutes. The results indicated that amplification of 1ng or less amount of DNA is successful in generating complete STR DNA profiles from most of the alcohol preserved tissues.

The Philisa® Streck Thermal Cycler is an innovative high speed PCR instrument that can lead to the improvement of laboratory efficiency and flexibility. It has industry leading ramp rates, thermal control and thin-walled plastic tubes that enable quick, reliable PCR. Shortening the lengthy amplification time in forensic DNA cases benefits laboratories without compromising accuracy and efficiency.