The Stability of Amplified Product in Forensic Casework During Long Term Storage

In the past few years, many forensic DNA laboratories have demonstrated that DNA can be obtained from a very small amount of biological material. In forensic casework it is possible that all material may be consumed during routine analysis and the only material remaining is the amplified DNA product generated from PCR analysis. Although other forms of DNA may be more stable than the amplified product, it may be necessary to utilize the amplified product for re-analysis or confirmation of the original analysis. The question is if that sample's stability is reliable enough to ensure accurate results upon re-testing after a period of time has elapsed.

An amplicon study was conducted by the Scottsdale Police Department Crime Laboratory to determine the stability of the amplified product using the Profiler Plus™ and COfiler™ kits manufactured by Applied Biosystems®. The purpose of this study was to verify that amplified DNA product would provide reliable results when retested. Samples from the sensitivity and mixed stain experiments used during our original validation were re-analyzed, along with current casework samples from our laboratory and samples from Arizona Department of Public Safety were also evaluated. Several factors were examined during this study such as length of time, storage conditions, amplification volumes, sensitivity, allelic dropout and artifacts. 25µl and 50µl reaction volumes were used to amplify the DNA using Profiler Plus™ and Cofiler™ kits and samples were analyzed on the ABI Prism 310 Genetic Analyzer™.

Although some samples showed a fluctuation in sensitivity over time, the same genetic profiles were obtained from all samples. However, the manner in which the samples were stored did have other effects on the samples. The samples from the sensitivity experiments showed allelic drop out at the 0.25ng, 0.125ng and 0.0625ng concentrations. These samples were 5 years

old, amplified in a 50µl reaction and stored at ambient temperature. The amplified DNA samples that were stored at ambient temperature also showed an increase in artifacts due to possible breakdown of the molecular dyes; however these artifacts did not effect the allele sizing performed by the GeneScan™/Genotyper™ and GeneMapper ID™ software. The same artifact was seen in the FGA locus of the samples at 279-280 base pairs and could size as a 19 peak. The peak shape of the artifact was broad and had a RFU value near 100. Peak broadening or tailing, often termed as plus a, was also observed at the D3S1358 and amelogenin loci. This is most likely due to the decrease in stability when stored at ambient temperatures.

Actual casework samples from our laboratory as well as Arizona DPS were re-analyzed at 3 months, 5 months, 1 year and 2 years. Samples that were refrigerated at -5° C demonstrated allelic drop out in the 50µl volume after one year but did not have the artifact in the FGA loci that were evident in those samples stored at ambient temperatures. Samples that were stored frozen at -15° C for 3 months, 5 months, 1 year and 2 years showed no signs of allelic drop out when using the 25µl amplification volume, provided there was at least 0.25ng of DNA present in the sample. Samples that were stored frozen at -15°C for 1 year demonstrated allelic dropout at the FGA, D21S11, D18S51 and D3S1358 loci when less than 0.25ng of DNA was present. When the injection time was increased to 10 seconds then the software made the appropriate allele calls.

In conclusion, amplified DNA can be a useful means of obtaining genetic profiles when the DNA extract and sample have been exhausted. Allelic drop out can occur when samples have less than 0.25ng of DNA and caution should be used when interpreting samples that contain less than 0.25ng of DNA. Amplified product that is stored frozen at -15° C is optimal to obtain the most reliable results. Although artifacts could appear over time, if the amplified product is improperly stored, the same profile can still be obtained for at least up to 5 years.