A122 Use of a Novel Enzyme to Prepare Samples for Forensic Analysis

Gregory A. Meyers, MS, Charles M. Kelly, PhD, and Roxana Ospina, BS, Commonwealth Biotechnologies, Incorporated, 601 Biotech Drive, Richmond, VA 23235; Nick Price, PhD, ZyGEM Corporation Ltd., 462 Stevens Avenue, Suite 304, Solana Beach, CA 92075; and Thomas Reynolds, BS, Commonwealth Biotechnologies, Incorporated, 601 Biotech Drive, Richmond, VA 23235*

The goal of this presentation is to demonstrate the use of a new enzyme for quick and easy preparation of DNA from various types of forensic samples.

This presentation will impact the forensic community by presenting data on the use of a novel enzyme for the treatment of various types of forensic samples for DNA analysis by STR typing. The procedure in general involves a brief digestion with an endopeptidase isolated from a novel extremophile organism from the Antarctic. The process can be carried out in a single tube with no transfers involved, thus minimizing chances for contamination or sample switches.

An expanding area in forensic DNA profiling involves databases of reference DNA profiles from known individuals. These profiles can be compared against others from crimes with no known suspect. Correspondence between a sample and the database allows linkage of an individual to a crime-scene and this may lead to a conviction. Reference DNA databases are usually compiled from past offenders or suspects (depending on jurisdictional legislation) and most often use buccal swabs to obtain reference profiles. There is therefore a need for a simple method that is easy to automate to help address the backlog of samples waiting for CODIS database testing.

For most DNA forensic analyses, the quality of DNA extracted directly affects the ability to obtain high quality forensic DNA profiles. The DNA extraction procedures commonly used in the forensic field tend to be time-consuming, costly, involve potentially toxic chemicals, and may involve repeated sample transfers which expose the sample to potential contamination – a feature that often leads to trepidation in forensic biology. Alternative strategies have been devised by many manufacturers (for example spin columns or magnetic beads), but these tend to be more expensive and still involve multiple transfer steps.

Data will be presented involving a novel DNA extraction method which can be carried out directly on forensic samples in a single tube with little or no sample transfer. The method is also easily automatable. The premise of these methods is the use of a broad specificity endopeptidase that can be added to forensic sample preparations and which remains minimally active until the sample reaches a temperature of 75°C. Upon reaching its activation temperature, the enzyme digests all proteins, including nucleases, that would interfere with downstream analysis. After this incubation, the preparation is brought to 95°C, whereupon the endopeptidase is totally and irreversibly inactivated. The resulting DNA solution can then be amplified by standard methods to obtain STR profiles.

This novel endopeptidase can be incorporated into kit form for sample preparation. Data obtained from such preparations will show results of DNA extraction from crime samples with optimized methods and formulations providing PCR-ready DNA in just over 30 minutes. Such preparations offer a gentle method which prevents release of inhibitors into the extracted DNA solution while maintaining a closed- tube state. This process works to minimise the risk of extraneous contamination by foreign DNA. Kits may be specifically designed to be compatible with common STR profiling kits and forensic genotyping methods.

Comparisons of the novel endopeptidase methods with the currently validated methods used for forensic samples were carried out. Sample types tested included buccal swabs, blood (whole and stain), and others, including low DNA copy number samples. Comparison of these isolation methods included several commercially available DNA quantitation kits and STR kits. The results of these comparisons will be presented.

Sample Preparation, STR Analysis, Novel Method

 ** Presenting Author*