



B59 Forensic STR Analysis of Bone Samples: Improvements in Extraction and Amplification With a Focus on High Throughput

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Attendees will be given an overview of the setup and optimization of the International Commission on Missing Persons' (ICMP) high throughput bone typing lab including development of a DNA extraction technique and selection of a STR typing kit.

Armed conflicts in the former Yugoslavia during the 1990s led to hundreds of thousands of deaths with up to 40,000 persons still missing. Most of these missing persons can only be identified through forensic DNA testing for a number of reasons, including: the time lapse since people died, the lack of dental or medical records, unreliable connections between personal effects and the recovered bodies, and commingled remains. To meet the challenge of DNA testing a large number of bone samples, the ICMP has developed a high throughput DNA testing system capable of obtaining STR profiles from up to a thousand bone samples per month.

Producing STR profiles from 8–11 year old bone samples is often challenging because the samples contain only low levels of DNA, the DNA present is frequently degraded, samples usually contain substances that are inhibitory to PCR reactions, and bacterial contamination may create complications. One of the limiting steps in the identification of individuals following mass fatalities is the ability to isolate a sufficient amount of quality DNA from bone samples. The most commonly used technique for isolation of DNA from bone samples involves organic extraction procedures. This method for isolation of DNA from 8–11 year bone samples was attempted. However, subsequent STR typing was only possible in approximately 30%–50% of the cases. While analysis of the DNA extracts obtained via organic extraction indicated DNA was successfully isolated in the majority of these cases, the frequent failure of subsequent STR testing was suggestive of amplification obstacles, such as the presence of inhibitory compounds.

In an effort to increase the success rate of bone samples undergoing STR analysis, a silica based extraction method has been developed. Using approximately 5–6 grams of a bone sample in this modified extraction method has produced a 90%–95% success rate with Promega's PowerPlex[®]16 STR. An even higher success rate of approximately 99% has been realized when this procedure is used to extract teeth. The silica-based method is faster, cheaper, safer, and easier than organic based extraction methods. Furthermore the silica based extraction method appears to be much better at removing the inhibitory compounds from bone and teeth samples.

Incorporation of the silica-based DNA extraction procedure has led to the development of a high throughput DNA testing system for skeletal remains. The ICMP's DNA laboratory in Sarajevo, Bosnia and Herzegovina currently tests 45 bone samples, in duplicate, per day. The high success rate of obtaining STR DNA profiles from bone samples has resulted in a significant increase in the rate of the identification of the missing in the former Yugoslavia with between 200–300 individuals currently being identified on a monthly basis.

STR, Silica, DNA