B159 High Throughput STR Testing of Tens of Thousands of 912-Year-Old Bone Samples From the Former Yugoslavia Using a Silica Based Extraction Method

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Attendees will be presented with a review of the International Commission on Missing Persons high throughput bone STR testing facility including optimization of extraction, amplification and the minimization of costs.

The results presented would have benefit to anyone performing STR analysis of difficult samples such as bone. These results may also be of benefit to other forensic projects whose goals are also to use DNA STR tests in order to confirm the identify of mortal remains. These results were obtained from possibly the largest mass identification process ever attempted.

The ICMP has developed a DNA-led identification effort to assist in the identification of the estimated 30,000 – 40,000 persons that went missing during the breakup of the former Yugoslavia. Due to the large number of missing persons and the lack of medical and dental records for this region the strongest piece of evidence that will lead to identification is a DNA STR profile. In the past large scale bone STR testing has not been feasible because of numerous limitations. Some of the limitations to performing large scale DNA testing on bone samples are the high costs, the high rate of failure in the testing process, the large number of loci needed to generate significant results, and the time required for such testing.

To increase the success rate of STR testing on bone samples a silica-based extraction method was developed and has been shown to be much more successful than that of the organic extraction method. To directly compare the DNA isolated by these two methods real time PCR was performed using the Applied Biosystems Quantifiler® kit on the ABI 7700 Sequence detection system. Preliminary results of this comparison show that the ICMP developed silica-based extraction protocol both isolates more DNA than the organic method as well as it reduces the levels of PCR inhibition that are often observed during amplification of DNA from bone samples. Further studies using the Quantifiler kit have allowed the optimization of PowerPlex® 16 and SeFiler® amplification reactions based on the estimated DNA content recovered from bone samples.

Using this silica based extraction system in combination with the Promega Power Plex® 16 system the ICMP has extracted nearly 22,000 bone samples for around 11,000 cases. Using this system at least 14 loci have been amplified for approximately 10,000 of these cases. For some cases where individual loci failed, the initial testing, attempts were made to amplify these loci using a single primer pair as a monoplex system has some advantages over the 16-plex system.

The average consumable reagent costs per case of bone STR analysis including all consumables is less than $100USD for duplicate bone sample extractions and $85USD for extraction of two teeth. This processing system in combination with the relatively inexpensive cost of labor in the former Yugoslavia has led to a rapid, relatively inexpensive system that is currently identifying 300 – 400 bodies per month.

DNA, STR, Real Time PCR

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