A36 Evaluation of DNA Extraction Efficiency

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After attending this presentation, attendees will understand the importance of evaluating extraction efficiency from a known amount of DNA, learning that the observed recovery value range was significantly lower (20 to 30 percent) than many reported extraction efficiency calculations using the number of full STR profiles produced.

This presentation will impact the forensic science community by bringing attention to the amount of DNA unrecovered during the extraction process. The evaluation of the amount of unrecovered DNA could lead to more efficient methods to recover higher percentages of DNA from the extraction and purification processes.

Forensic DNA typing requires a specific quantity of input DNA (typically 0.5 – 1.0 nanograms) to generate an optimal Short Tandem Repeat (STR) profile. For reference samples, the amount of DNA collected on a standard buccal swab or blood punch is generally in excess of that which is needed for testing (on the order of hundreds of nanograms (ng)). Typically, extraction efficiency is evaluated by determining the number of samples that produce a full STR profile divided by the total number of samples processed. Less attention has been paid to the amount of DNA unrecovered during the extraction process from the original sample. Determining the amount of unrecovered DNA after the extraction process requires the original amount of DNA to be known prior to extraction, which is not the case in reference or casework samples. The importance of evaluating the theoretical yield versus the functional yield is in cases when the initial amount of available DNA is low. Within the extraction process, a majority of the DNA sample is lost, which has minimal impact on reference samples because enough DNA is recovered for an STR profile, but can have a significant impact non-reference samples within a laboratory. In these cases, it would be beneficial to obtain an extraction recovery that is closer to the theoretical yield than the functional yield. Evaluating the amount of unrecovered DNA could lead to more efficient extraction methods to recover higher percentages of DNA from the extraction and purification processes.

Extraction efficiency experiments were conducted to evaluate the percentage of DNA recovered through two extraction methods: a salting out procedure and use of the Qiagen EZ1 Advanced XL extraction robot with the DNA Investigator kit.[1] Three DNA sources were tested using varying initial amounts of human cells, previously purified and highly characterized DNA, and liquid whole blood. Controlled amounts of DNA from the three DNA sources were absorbed onto cotton buccal swabs, specimen collection paper, and FTA paper. The cells were spotted onto the swabs and paper in a PCR-compatible buffer suspension consisting of a 1.0 % BSA, 0.9 % NaCl, and 10 mM TRIS solution. Theoretical DNA quantities were estimated in total nanograms of DNA and applied to estimate a recovery percentage for each extraction. Human cells were quantified using a Coulter Counter and suspended within the PCR compatible buffer for each of the appropriate concentrations. The white blood cell count of a healthy individual ranges between 3.5 million and 10.5 million cells per milliliter, and a value of 7.0 million white blood cells per milliliter was used to determine theoretical DNA quantity for all whole blood samples.[2] The theoretical DNA series examined ranged from 24ng to 1800ng for all sample types. All extracted samples were quantified with Life Technologies Quantifiler Human DNA quantification kit in replicates of two. Results indicated that extraction efficiency ranged from 20% to 30% and recovery was independent of extraction method and DNA source. The observed recovery value range was significantly lower than many reported extraction efficiency calculations relying solely on the number of full STR profiles obtained.

References:

1. S.A. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucl. Acids Res. 16 (1988) 1215.
2. Complete Blood Count. Mayo Clinic. http://www.mayoclinic.com/health/complete-blood-count/MY00476/DSECTION=results. Accessed July 31, 2012.

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