B136 Strategies for Low Copy Number (LCN) DNA Analysis

Virginia L. Raker, BS, Erin K. Hanson, MS, and Jack Ballantyne, PhD, National Center for Forensic Science, University of Central Florida, Department of Chemistry, PO Box 162367, Orlando, FL 32816-2367*

After attending this presentation, attendees will understand various strategies that would enable samples containing low copy numbers of starting DNA template to be DNA profiled

The forensic community may be able to use the LCN typing strategies presented to obtain DNA profiles from samples containing low copy numbers of starting DNA template.

Low copy number (LCN) analysis is an approach that involves the examination of minute quantities (i.e., <100 pg) of DNA template. Even though genetic profiles can readily be obtained from body fluid stains when enough DNA is present for analysis, there are times when a genetic profile cannot be obtained because of the presence of too little DNA for analysis. When minute quantities of DNA are encountered in casework, LCN typing can provide a means by which a genetic profile can be obtained. In an attempt to provide casework laboratories with strategies to increase their DNA typing success rate by being able to routinely employ LCN methods, various LCN typing strategies were examined.

Initial experiments concentrated on the ability of increasing the PCR cycle number, using the D1S80 PCR-VNTR system as a model, in an attempt to type single or few cells. Cycle numbers ranged from the standard 30 cycles up to 50 cycles. Results indicate that increasing the cycle number to 35 cycles often resulted in the ability to type single or few (<5) cells. Increasing the cycle number beyond 35 resulted in the formation of non-specific amplification products that may obscure the presence of the true alleles. Concomitant with the ability to type single or few cells, a loss of heterozygosity (allelic drop-out) was observed.

Several whole genome amplification (WGA) methods were evaluated for LCN analysis in order to permit increased sensitivity of analysis down to a singe cell equivalent. The WGA methods tested were primer extension PCR (PEP), degenerate oligonucleotide primed PCR (DOP), and multiple displacement amplification (MDA). PEP uses a mixture of random 15 base oligonucleotide primers to prime and subsequently amplify the whole genome (or a large percentage of it) prior to subsequent genetic analysis while DOP uses a single degenerate primer. MDA is an isothermal process that employs random hexamer primers to amplify the whole genome.

Cell suspensions were first subjected to WGA analysis using PEP, DOP, and MDA. Aliquots of the products were then re-amplified using primers specific for the D1S80 PCR-VNTR locus. Cycle numbers ranged from the standard 30 cycles to 50 cycles. The results obtained are promising. It appears that WGA products from PEP and DOP can be used to increase the sensitivity of D1S80 analysis from a few cells to one cell-equivalent. Results indicate that WGA products from MDA can be used to type a few (<5) cells, but the sensitivity of D1S80 analysis is not greatly increased using this WGA method. Allelic drop-out was observed with each WGA method tested—PEP, DOP, and MDA—as expected. Allelic drop-in and non-specific amplification products were also observed. However, the extraneous alleles that were detected did not interfere with genetic typing of the samples. The preceding WGA analyses used the D1S80 PCR-VNTR system as a model; the WGA assays are being applied to autosomal and Y-STR typing systems. Detailed results will be presented including an evaluation of the efficacy of these methods for casework specimens.

Low Copy Number (LCN), Increased Cycle Number (ICN), Whole Genome Amplification (WGA)

 ** Presenting Author*