

## SETTING INTERPRETATION THRESHOLDS AND RESULTS WITH LOW-LEVEL DNA ANALYSIS

Carolyn R. Hill and John M. Butler

National Institute of Standards and Technology, Applied Genetics Group, Gaithersburg, MD 20899-8312

The term Low Copy Number (LCN) DNA is typically used when there is less than 100 - 125 pg of genomic DNA present in a sample [1,2]. More and more labs are attempting to process lower amounts of DNA without realizing the consequences of doing so. STR typing kits will generally fail to amplify all of the loci present or even one or both alleles present within a locus at these low levels of DNA. Partial incorrect profiles are generated that can be misleading without taking additional precautions including replicate testing [3,4]. In these cases, there are too few copies of the DNA template to provide reliable polymerase chain reaction (PCR) amplicons, causing preferential amplification to occur [5]. Next generation manufacturers' kits are being made more sensitive with improved PCR master mixes and more robust DNA polymerases. This can potentially lead to labs pushing the envelope and getting results that may not represent the true DNA profile of the originating source due to stochastic effects including allele dropout or drop-in.

We have performed multiple LCN experiments to evaluate three different samples that are heterozygous at every locus in the AmpFISTR Identifier™, AmpFISTR MiniFiler™ (Applied Biosystems, Foster City, CA) and PowerPlex 16 HS (Promega Corporation, Madison, WI) PCR amplification kits. Completely heterozygous samples were used in order to evaluate peak height ratios and potential imbalance due to stochastic effects (as compared to 9947A which is often used but has many homozygous loci). Each sample was tested in triplicate at multiple concentrations, including several considered to be LCN amounts (1 ng, 200 pg, 150 pg, 125 pg, 100 pg, 50 pg, and 10 pg) and at different PCR ranging from 28 to 34 cycles [3,6]. They were tested in triplicate to determine the consensus profile, where an allele cannot be scored (considered real) unless it is present at least twice in the triplicate samples [2,3,6,7]. The heterozygote peak height ratios (PHR) were calculated and compared at different concentrations and PCR cycling [2,4]. Results will be shown with different multiplex kits. In addition, post-PCR purification was performed on some of the samples to evaluate the impact of salt removal on signal strength and data interpretation. Post-PCR purification requires a change in interpretation threshold. Therefore, thoughts on setting interpretation thresholds to avoid stochastic effects will be described. The value of anchoring DNA quantitation results to a calibrated reference material will also be discussed.

### REFERENCES

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