Setting Interpretation Thresholds and Results with Low-Level DNA Analysis

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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]. Efforts to enhance signal include longer injection with capillary electrophoresis, salting out from the amplified product, reduced volume polymerase chain reaction (PCR), increasing the number of PCR cycles, and increasing the enzyme concentration. More and more labs are attempting to process lower amounts of DNA, many 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. 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 (PCR) amplicons, causing preferential amplification to occur [5]. Next generation manufacturers’ kits are being made more sensitive with improved PCR masters, 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 LCM experiments to evaluate two different samples that are heterozygous at every locus in the AmpFISTR Identifier™ (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 99447A which is often used but has many homozygous loci). Each sample was tested with 10 replicates at multiple concentrations, including several considered to be LCN amounts (1 ng, 100 pg, 30 pg, and 10 pg) and at different PCR ranging from 28 to 34 cycles [3,6]. They were tested with 10 replicates to determine the consensus profile, where an allele cannot be scored (considered real) unless it is present at least twice in the replicate samples [2,3,6,7]. The heterozygote peak height ratios (PHR) were calculated and compared at different concentrations and PCR cycling [2,4]. In addition, 1:3 and 3:1 mixture samples at LCN total DNA amounts (100 pg) were evaluated and compared in 10 replicates. Results are shown with different multiplex kits. 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.

### Stochastic Thresholds for Interpretation

Two thresholds are often set for analysis of STR typing data. The analytical threshold (typically set at around 50-100 RFUs) reflects the instrument sensitivity, which is impacted by baseline noise in collected data. The analytical threshold helps determine what is a true peak signal—and thus a potential STR allele. The stochastic threshold (typically at 100-200 RFUs) helps determine what is reliable PCR data and is impacted by assay and CE injection parameters. When a peak is above both thresholds (high confidence), the peak is considered to be a STR allele. In addition, 1:3 and 3:1 mixture samples at LCN total DNA amounts (100 pg) were evaluated and compared in 10 replicates. Results are shown with different multiplex kits. 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.

### LCN Sensitivity Data and Results with Identifier™ and PowerPlex® 16 HS:

#### Experimental Design

- **Pristine DNA Samples**
  - 2 samples (mixtures) were made from the diluted samples.
- **Low Template DNA Amounts**
  - 2 samples were made at 1 ng DNA amount, 31 cycles.
- **Increased Cycle Number**
  - 2 samples were made at 31 cycles.
- **Replicates**
  - 10 replicates were made for each sample.

#### Example Profiles

- **identifier, 31 cycles**
  - MT 97150_100 pg
  - MT 97150_10 pg
  - MT 97150_30 pg
  - MT 97150_1 pg
- **PP16HS, 34 cycles**
  - Penta E
  - Penta F
  - Penta M
  - Penta X
  - Penta A
  - Penta D
  - Penta S
  - D13S317
  - D16S539
  - TH01
  - D5S818
  - D7S820
  - D8S1179
  - CSF1P
  - TH01
  - D5S818

#### Peak Real, Can

- **allele dropout**
  - 100 pg
  - 30 pg
  - 10 pg

#### Consensus Profiles for Optimal Results

- **Typically 2 - 3 PCR amplifications**
- **An allele is usually not scored** (considered real) unless it is present at least twice in replicate samples
- **Challenges of Low Level DNA Mixtures**
  - 2 samples that are heterozygous at all loci were mixed in 1:3 and 3:1 ratios with a 100 pg total amount of DNA and were tested with Identifier (31 cyc) and PP16HS (31 cyc) in 10 replicates.

#### Sensitivity Comparisons

- **LCN loci contained within each kit are depicted across the X-axes, and input DNA amount in ng and replicate results is shown on the Y-axes.**

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### References