QIAGEN® MINELUTE PCR PURIFICATION INCREASES SENSITIVITY IN FOUR MULTIPLEX PCR KITS

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Two means determine the sensitivity of STR detection: total relative fluorescence units (RFU) and alleles detected. Several methods can be used to increase the sensitivity of low-copy number (LCN) DNA samples, such as increasing the number of PCR cycles; increasing injection time or voltage[1]; increasing the amount of PCR product analyzed; and removing primers, buffers, and non-amplicon products from the PCR product. QIAGEN® MinElute PCR Purification uses a column to bind, wash and elute amplified PCR product to remove excess primers and PCR components[3] leaving the sample cleaner and more concentrated. A previous study suggests that post-PCR purification of the amplified product with QIAGEN® MinElute can increase the sensitivity to effectively detect DNA profiles from <100 pg of DNA; but typical problems for LCN samples such as allele dropout, increased stutter, and sporadic contamination still exist.[2] However, we have shown that QIAGEN® MinElute increases the sensitivity as measured by total RFU and alleles called with four multiplex PCR kits PowerPlex® 16, AmpFlSTR® SGM Plus®, AmpFlSTR® YFiler™, and AmpFlSTR® MiniFiler™ at the North Louisiana Criminalistics Laboratory (NLCL). A working concentration of 0.1 ng/µL of female and male DNA was used to prepare DNA samples for amplification and serially diluted two-fold to yield a final template of 1.0, 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016 ng in 10 µL of TE^{-4} buffer. Each amount of DNA was amplified for each of the systems and subjected to QIAGEN® MinElute PCR purification. Compared to samples not cleaned QIAGEN® MinElute PCR purification, there is an overall increase in total RFU and alleles detected for DNA amounts <125 pg. However, for template amounts >125 pg, there are considerable artifacts and stochastic effects which include allele drop in, increased stutter, and pull-up from too much amplified product. Thus, the effectiveness of QIAGEN® MinElute PCR purification is dependent on the initial amount of DNA input and does not produce useable results for DNA amounts greater than 125 pg. Non-probative samples for PowerPlex® 16 and AmpFlSTR® SGM Plus® amplification kits show an increase in sensitivity as measured by both total RFU and alleles detected for two sets of samples; those with no initial profile and samples with a partial profile. The use of QIAGEN® MinElute PCR purification for amplified PCR product results in an increased sensitivity for low copy number DNA samples and therefore will increase the ability to detect alleles in casework samples.