B64 Simpified Low Copy Number (LCN) DNA Analysis by Post PCR Purification

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After attending this presentation, attendees will understand the effect of various post PCR purification methods on the sensitivity of fluophore-based allelic detection using capillary electrophoresis; learn how to perform low copy number DNA analysis (< 100 pg) using 28 cycle amplification; and learn how to enhance weak DNA samples (> 100 pg) to obtain full DNA profiles.

This presentation will impact the forensic community and/or humanity by demonstrating methods that will allow for genetic information to be obtained from forensic DNA samples that previously would not have been detected due to low DNA template. An immediate application can be made in forensic case working laboratories for the enhancement of weak DNA samples. In addition, a bona fide alternative to LCN analysis is described which may be utilized in lieu of or in conjunction with increased amplification cycle LCN analysis. This method may prove to have greater allele fidelity than increased amplification cycle as indicated by negative amplification controls.

Frequently evidentiary items contain an insufficient quantity of DNA to obtain complete or even partial DNA profiles using standard forensic gentotyping techniques. This presentation explores the effect of increasing PCR sensitivity without increased amplification cycles. Standard 28 cycle amplification is followed by purification of the PCR product. Un-reacted reaction components are removed from the amplification mix prior to capillary electrophoresis thus preventing their competition with amplicons during electrokinetic injection. Here, various methods of post PCR purification are evaluated for their effects on the sensitivity of fluophore-based allelic detection. A method of post PCR purification is described which increases the sensitivity of standard 28 cycle PCR such that weak samples (> 100 pg) can be enhanced to obtain full DNA profiles. With minor modification this method of post PCR amplification increases the sensitivity of standard 28 cycle amplification such that low copy number DNA templates (< 100 pg DNA) can be analyzed. Full profiles were consistently obtained with as little as 20 pg template DNA and significant allelic data was generated with as little as 5 to 10 pg DNA without increased cycle number. In mock case type samples with dermal ridge fingerprints, genetic profiles were obtained by amplification with 28 cycles followed by post-PCR purification whereas no profiles were obtained without purification of the PCR product. Allele drop out, increased stutter, and sporadic contamination typical of LCN analysis were observed; however no contamination was observed in negative amplification controls. The effects of low copy number DNA analysis by post PCR purification on stutter and heterozygote peak imbalance are also described. In addition, guidelines for the application of post PCR purification to amplified DNA samples are presented.

PCR Purification, Low Copy Number, MinElute

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