B104 Genotyping Challenging DNA by the Isolation of Polymerase Chain Reaction Products (IPCRp)

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The goal of this presentation is to demonstrate the application of the IPCRp method for genotyping challenging DNA samples, in particular, touch and low copy DNA samples.

This presentation will impact the forensic science community by increasing competence when genotyping challenging DNA samples, such as touch and low copy DNA.

Genotyping field-collected samples of low quantity amplifiable copies or Low Copy Number (LCN) DNA has been a considerable challenge primarily due to the constraints of the PCR reaction itself in which the amount of the amplified targeted DNA at the end of the PCR cycle process is directly proportional to the amount of the template DNA at the start of the reaction. Increasing the number of cycles or going through another cycling process, as in the case of the nested PCR method, would increase the amount of a PCR product. Optimizing the PCR reaction for increased cycle number requires advanced technical skills and specialized reagents; however, even when done properly, high PCR cycle reaction creates additional problems due to increased stutter and background noise, the most important factors that have an adverse effect on the genotyping of LCN DNA. The IPCRp genotyping method reportedly can increase the detection levels more than 10-fold compared to classical PCR, does not require increased cycle number, and eliminates background noise in the process of genotyping.

The IPCRp method was used for genotyping touch DNA samples. Fingerprints from a stainless steel bar were swabbed and DNA was extracted by QIAGEN® Micro Kit, followed by IPCRp amplification and genotyping on a Capillary Electrophoresis (CE) genotyper platform. The 6-Plex PCR kit was designed according to the IPCRp method protocol with both forward and reverse primers labeled with fluorescent dye and biotin, respectively. The kit simultaneously amplified THO1, FGA, CSF, D21, TPOX, and D7 loci. Following the PCR, the double-stranded PCR product was captured on a streptavidine plate, washed, and only dye-labeled targeted DNA in single-stranded configuration was released and loaded to the genotyping platform. The loading quantities of the PCR product into streptavidin plates and the CE instrument were optimized. With the IPCRp amplification/genotyping method, a full genotype was obtained out of 10pg of template DNA when 12µl of captured targeted DNA was loaded to a 310 Genotyper®.

The full profile was obtained from all touched DNA samples, the stutter levels were not increased, and there was considerable reduction in background noise. Results from genotyping touch DNA samples and low copy number DNA will be presented. It is recommended that the IPCRp method be used in every case with genotyping challenging samples.

Reference(s):

1. Dimsoski P., Woo S. *Novel method for isolating single stranded product.* US Patent app. 10/723,388, 2003.
2. Dimsoski P., Woo S. Increasing detection of polymerase chain reaction (PCR) by isolation of PCR products (IPCRp). *Croat Med J.* 2005; 46(4):619-621.

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