TARGETED GENOME AMPLIFICATION TO SUPPORT STR PROFILING OF LOW COPY NUMBER DNA SAMPLES

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In recent years, whole genome amplification (WGA) techniques have been used to enrich the genomic material in a sample so that low-level DNA can be successfully amplified. As it has been applied in human forensics, WGA is, essentially, the first step in a genome-wide nested PCR protocol in which primers bind randomly across the genome, generating the material to support a subsequent locus specific PCR.

However, there are a number of problems commonly associated with WGA protocols, including stochastic allele drop-out, strand slippage (or stutter), and the reduction of product length with successive rounds of amplification. A less talked about issue is that WGA unnecessarily complicates a sample by increasing the quantity of non-target DNA, which competes for resources in the subsequent profiling reaction. For standard forensic applications, we know the targets of interest – the sixteen loci contained in an STR profiling multiplex. Therefore, if we could selectively amplify only the relevant STR regions such that they overwhelm the areas of non-interest then we could better support multiplex-specific amplification from limited starting material.

We have developed a novel WGA technique, called targeted genome amplification (TGA), which selectively amplifies only the relevant STR regions. Primer sequences for Promega's PowerPlex16 were used to define the regions of interest. Novel primers were designed flanking the loci, each designed to amplify a region uniform in size in an effort to eliminate preferential amplification of smaller alleles. A proprietary modification was made to each primer to promote homogeneous amplification of all alleles while significantly reducing stutter.

A seven member subset of the PowerPlex 16 multiplex was selected for use in the optimization of reaction conditions. Using this system, we have been able to amplify a complete seven locus profile, free from excess stutter, from as little as 25 pg DNA (~4 genome equivalents), with partial profiles from 12 pg DNA (~2 genome equivalents). Future studies include the addition of the remaining PowerPlex 16 loci to the mix, as well as the additional markers included in Applied Biosystems Identifiler (D19 and D2), so that the technique is of use upstream of either system. The protocol will be further tested using whole cells, isolated by laser capture microdissection. Once the assay is fully optimized, it should be possible to accommodate the amplification of different types of markers, i.e. Y-chromosome or mitochondrial DNA, by modifying the primer sets.