B50 Application of a Linear-Targeted Approach in Multiplex Amplification of the Mitochondrial Genome

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After attending this presentation, attendees will have a better understanding of a linear-targeted approach designed to increase template DNA in low copy samples. Moreover, implementation of the linear approach in multiplex amplification and Massively Parallel Sequencing (MPS) of the whole mitochondrial genome will be presented.

This presentation will impact the forensic science community by providing an avenue to maximize data recovery from low quality samples.

In cases where limited or degraded nuclear DNA precludes the use of capillary electrophoresis to obtain a reliable Short Tandem Repeat (STR) profile, forensic scientists often turn to the mitochondrial genome due to its higher copy number per cell. The emergence of MPS in forensic science has enhanced the capability to recover extensive sequence information, thereby increasing the power of discrimination in mitochondrial DNA (mtDNA). Furthermore, MPS provides the breadth and depth of coverage necessary to detect low-level heteroplasmic variants across the mitochondrial genome (mtGenome) with a relatively small initial DNA input.

Though mtDNA may afford a more viable option when sample quality is questionable, there are instances where DNA yield remains too low to deliver a reliable haplotype. Polymerase Chain Reaction (PCR) -based techniques have been successful in increasing DNA yield of low-copy number samples; however, due to the exponential nature of PCR, increasing PCR cycle numbers can exacerbate stochastic sampling effects, especially in diploid nuclear DNA.[1,2] One method suggested to help alleviate this problem is targeted non-exponential amplification.[3] A Single Strand Extension Assay (SSEA) seeks to boost copy number prior to traditional analysis by employing a linear-targeted amplification approach. Briefly, samples are divided and amplified separately in two reactions with either a forward or reverse primer. Similar to PCR, SSEA includes denaturation, annealing, and extension steps, but with either forward or reverse primer in the reaction, only one additional copy of the product is produced after each cycle. Following SSEA, products from each reaction are pooled and sufficient template DNA is available for subsequent PCR analysis. Due to its haploid nature, the mtGenome provides an optimal target for evaluating the utility of SSEA, as the stochastic effects associated with diploid genomes (e.g., heterozygote imbalance, allele drop-out) will be excluded.

The utility of SSEA on mtDNA was initially tested for the hypervariable region. Commercially available DNA (HL-60) and DNA isolated from telogen hairs were diluted to low-template levels and amplified in separate forward and reverse reactions. Amplification success was evaluated on the Agilent® 2100 Bioanalyzer and absence of primer dimer was confirmed. Forward and reverse products were then combined and subjected to a second round of traditional PCR, prepared for MPS using Nextera® XT kit, and run on Illumina® MiSeq®. Preliminary results indicate that performing SSEA on low-template samples prior to traditional PCR provides sufficient target DNA for MPS.

To further test the effectiveness of SSEA, a previously developed low-volume multiplex PCR assay that amplifies ~350bp-650bp fragments around the mtGenome was employed. The multiplex assay has successfully amplified telogen hair and bone samples with minimal DNA input (fewer than 1,500 copies) and average MPS coverage ranges from 11,530 to 23,239. To implement SSEA using the multiplex assay, primers from either the heavy or light strand were carefully selected and extension performed separately for each of three multiplex reactions. Heavy and light strand products were then combined and subjected to a second round of traditional PCR, prepared for MPS using Nextera® XT kit and run on Illumina® MiSeq®.

The SSEA combined with the low-volume multiplex assay provides a viable option for maximizing data recovery from low-template DNA samples. Future research will focus on applying SSEA to other types of forensically relevant samples as well as evaluating SSEA performance in nuclear DNA using MPS.

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Reference(s):

1. Budowle B., Eisenberg A.J., van Daal A. Validity of low copy number typing and applications to forensic science. *Croat Med J*. 2009; 50: 207-17.
2. Caddy R., Taylor G., Linacre A. A review of the science of Low Template DNA analysis. Executive Summary, Home Office, United Kingdom; 2008.
3. Grisedale K., van Daal A. Linear amplification of target prior to PCR for improved low template DNA results. *BioTechniques*. 2014; 56(3): 145-7.

Linear Amplification, Mitochondrial Genome, Multiplex Amplification

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