REVERSE COMPLEMENT PCR (RC-PCR) SYSTEM FOR ANALYSES OF HIGHLY DEGRADED DNA SAMPLES.

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Several human identification marker systems are available and validated for forensic DNA typing purposes. However, a number of forensic samples, e.g., human remains and touch evidence, do not contain sufficient quantity and quality DNA to be typed with these current methodologies. Several studies have reported that DNA fragments in highly degraded samples can be as short as approximately 50 bases in length. Only a limited number of DNA markers, if any, may be detected with such short fragments. SNPs, which are abundant in the human genome, can, in theory, be amplified in shorter amplicons (~50-100 bps) than those of current forensic markers. But developing a robust multiplex of short amplicons can be challenging. Two advances now may make it possible to analyze highly degraded DNA: Reverse Complement PCR (RC-PCR) and massively parallel sequencing (MPS). RC-PCR is a novel one-step PCR target enrichment and library preparation method for MPS, which is performed in a single, closed-tube system. The method utilizes two reverse-complement, target-specific primer probes (RC probes) with a universal tail, Illumina universal i5 and i7 indexes, and sequence adapters. Instead of targeting the flanking region of a DNA marker directly, the RC probe targets the Illumina index primer via the universal tail. During RC-PCR, a 3′-end blocker prevents extension of the RC probe while functional, target-specific index primers are generated and extended. Subsequently, the library, which requires only a few hours for preparation, is ready for pooling, purification, and sequencing. Lastly, the RC-PCR process reduces library preparation manipulation steps, including the opening and changing of the tubes and sample transfer, which reduces the chance of contamination and DNA loss. A multiplex of 85 identity SNPs (most with amplicon lengths of 50-100 bp) has been developed, of which 33% of the SNPs are contained within amplicons that are approximately 50 bp long. Thus, highly degraded samples may be typed with commonly used identity SNPs. Several studies have been performed on this multiplex: sensitivity of detection, allele/locus call rates, heterozygote balance, concordance, and noise. The results of this study support that the method and panel are a substantial improvement compared with most currently used systems.