REVERSE COMPLEMENT PCR: A NOVEL SYSTEM FOR THE TARGETED CAPTURE OF IDENTITY SNPs FOR TYPING HIGHLY DEGRADED DNA

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Reverse Complement PCR (RC-PCR) is a novel one-step PCR target enrichment technology for amplifying short fragments of DNA. It allows for simultaneous amplification and tagging of a targeted sequence construct in a single, closed tube assay. The library-prepared products are suitable for massively parallel sequencing (MPS). A human identification (HID) RC-PCR panel was designed targeting 27 human identity single nucleotide polymorphisms (SNPs) contained within DNA fragments approximately 50 bases in length. The RC-PCR technology uses four probes: two target-specific primer probes (RC probes) with a universal tail and two universal barcoding primers. These barcoding primers contain sample-specific barcodes, the Illumina i5 or i7 index and adaptor sequences, and the complement sequence to the tail of the RC probes. The target-specific primers do not directly target the region of interest, but rather are the reverse complement and are blocked at the 3′ end to halt extension. In a single reaction, a complete sequencing construct is generated that includes everything necessary for MPS library preparation. Concordance studies examining a population of Caucasian males (N=50) produced consistent genotypes with known profiles. The system's ability to tolerate known PCR inhibitors frequently encountered in forensic casework (calcium, collagen, humic acid, and hematin) was evaluated. Hematin was the only inhibitor that had a negative impact on PCR yield. The other inhibitors were not effective until the highest concentrations were introduced. These findings may support the utility of the RC-PCR system with characterization of skeletal remains. In a sensitivity study, the RC-PCR system was able to detect the majority (at a minimum 83%) of alleles at a DNA input of 60 pg. Highly degraded samples (problematic population samples and casework samples from closed cases) were analyzed and compared with ForenSeq™ DNA Signature Prep Kit (Verogen, Inc.; San Diego, CA) MPS and capillary electrophoresis short tandem repeat (STR) analyses, respectively. A higher percentage of alleles were obtained for all samples using the RC-PCR technique. The success is likely due to the small amplicon size of approximately 50 bases which favors analysis of degraded DNA and is a more efficient amplification than larger amplicons by other approaches. The RC-PCR method appears to have substantial sensitivity of detection and robustness within fragments only 50 bases in length indicating that it may perform better than current methods in the analysis of degraded DNA.