

VALIDATION OF THE GLOBALFILER™ EXPRESS PCR AMPLIFICATION KIT FOR THE DIRECT AMPLIFICATION OF REFERENCE DNA SAMPLES

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The GlobalFiler™ Express PCR Amplification Kit (Life Technologies) was designed specifically to process reference DNA samples and includes the expanded STR loci proposed by ENFSI/EDNAP and the FBI. The kit uses 6-dye chemistry to enable typing of 21 autosomal STRs, a Y-STR, a Y chromosome indel, and the sex-determining marker amelogenin. In addition to its extensive multiplexing capability, the kit also incorporates a rapid amplification time of approximately 40 minutes.

The Life Technologies Center for Forensic Excellence (CFE) at the University of North Texas Health Science Center functions as a training facility for high throughput reference DNA sample processing. The CFE currently uses the Identifiler® Direct PCR Amplification Kit as the main multiplex assay in its workflow; however, considerations have been made to transition to the GlobalFiler™ Express PCR Amplification Kit for the typing of reference DNA samples. Prior to implementation and pursuant to laboratory guidelines and SWGDAM Quality Assurance Standards for DNA Databasing Laboratories, internal validation of the kit was conducted. The following parameters were considered as they apply to database-type samples: cycle sensitivity, reproducibility, precision, minimum threshold, contamination assessment, concordance, sensitivity of detection and stochastic effects. Samples consisted of blood and buccal samples on various collection media. To reduce manual punching and pipetting, several robotic instruments were used including the BSD-600 DUET semi-automated punching system, the Hamilton MICROLOAB® STARlet liquid handling platform and the TECAN Freedom EVO® 75 liquid handling platform. PCR products were separated by capillary electrophoresis using the ABI 3500xL Genetic Analyzer and data analysis was conducted using GeneMapper® ID-X software. Optimal amplification and injection parameters for a 1.2mm punch from blood and buccal samples were determined to be 27 and 28 cycles, respectively, combined with a 12 second injection time. From the concordance check, samples amplified under these conditions with GlobalFiler™ Express and previously validated conditions with Identifiler® Direct were determined to be comparable in terms of profile quality and sensitivity. Overall, the results show that the GlobalFiler™ Express PCR Amplification Kit can be used to obtain reliable typing of blood and buccal reference DNA samples.