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Developmental validation of the GlobalFiler® express kit, a 24-marker STR assay, on the RapidHIT® System

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ABSTRACT

Rapid DNA typing provides a transformative solution to help forensic laboratories and law enforcement agencies solve and prevent crimes. The RapidHIT® System is a fully integrated instrument with a simplified user interface enabling an operator to run the system and obtain a DNA profile from a sample in less than two hours. The integration and developmental validation of the NDIS-approved 24 loci GlobalFiler® Express kit expands the capabilities of the RapidHIT System to increase discrimination power, reduce adventitious matches, and improve cross-border data sharing capabilities. Developmental validation studies were performed according to the SWGDAM guidelines and tested several critical areas of performance including three sensitivity studies, inhibited samples, thermal cycling parameters, and cross-contamination. Validation studies indicate that the optimized PCR parameters and sensitivity of the system is capable of generating STR profiles from buccal or blood swab reference samples. Results were concordant with genotypes produced using standard bench thermal cyclers and capillary electrophoresis platforms. Furthermore, swabs can be retrieved from the system and re-run or reprocessed with traditional bench chemistries, e.g. Y-STRs, to gain additional information. Our results demonstrate that the GlobalFiler Express assay run on the RapidHIT System is reliable for generating profiles from reference samples after forensic review.
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1. Introduction

The RapidHIT System is a fully automated sample-to-DNA profile instrument for short tandem repeat (STR) based human identification [1,2]. This system integrates routine laboratory steps by performing cell lysis, DNA isolation, STR amplification, electrophoretic separation, fluorescent detection, and data analysis to generate DNA profiles in under two hours. Previously, PowerPlex® 16 HS chemistry (Promega Corp., Madison, WI), a 16 marker assay, was validated on the RapidHIT System [3,4]. However, the Federal Bureau of Investigation (FBI), European Network of Forensic Science Institute (ENFSI) and European DNA Profiling Group (EDNAP) have all agreed to the addition of STR loci to the European Standard Set (ESS) and to the core CODIS loci to increase cross-border data sharing, increase discrimination power, and reduce adventitious matches [5–8]. Furthermore, the Prüm treaty [9,10] was enacted into European Union legislation which requires member states to submit the five additional loci that are part of the new expanded 12 ESS [11]. This led manufacturers to develop and commercialize products that include all the required and/or recommended loci as requested by ENFSI, EDNAP and the core CODIS Loci Working group [12,13].

The GlobalFiler Express PCR Amplification Kit from ThermoFisher Scientific (Waltham, MA), an NDIS approved chemistry, contains all the required and recommended loci [6,8]. The kit contains 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338) and 3 sex determining markers (Amelogenin, DYS391, Y-indel). Use of fluorescent six-dye technology enables the amplicon sizes to be less than 400 bp (except SE33, 442 bp). To support the community worldwide, increase level of discrimination, facilitate international DNA profile comparison, and reduce risk of adventitious matches, the GlobalFiler Express assay was integrated and validated on the RapidHIT System.

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The developmental validation experiments presented here were performed according to the quality assurance standards issued by the Director of the FBI [14] and the revised guidelines published by the Scientific Working Group on DNA Analysis (SWGDAM) [15]. The results confirm the reliability of the NDIS-approved GlobalFiler® Express assay on the RapidHIT System for generating DNA profiles from reference samples. The profiles can be uploaded after forensic expert review to national and international databases once laboratories have completed their internal validation.

2. Materials and methods

2.1. DNA samples

Buccal swab samples were collected from consenting donors using 3 inch cotton-tipped swabs from Puritan Medical Products Company (Guilford, ME). Each donor was instructed to swipe the inside of the cheek ten times and contribute swabs daily to generate aged swabs for stability studies. After buccal collection, swabs were returned to their original paper package labeled with the date and an anonymized identification number, then stored at room temperature in a file cabinet.

Buccal swabs from all donors (138 males, 102 females) that had been collected over the past year were used to generate a reference database. This donor pool consisted of current employees, employee family members and former employees. The swabs were prepared using a slight modification of the GlobalFiler Express buccal swab protocol [16]. Briefly, 300–400 μL of Prep-N-Go™ Buffer (ThermoFisher Scientific) was added to 1.5 mL Eppendorf tubes. The cotton swab was inserted into the tube with buffer and incubated at 70 °C (vs. 90 °C) in a heating block for 15 min. The lysates were used to obtain an STR profile as described below.

The human male fibroblast cell line HTB-157 (ATCC, Manassas, VA), designated 1000 M, was used to prepare positive control swabs. The human embryonic palatal mesenchymal (HEPM) cell line CRL-1486™ (ATCC, Manassas, VA), designated 1000 F, was used for the mixture study. Cell culture optimization and scale up was performed under contract by Aragen Bioscience (Morgan Hill, CA), and cells were stored in 90% FBS, 10% DMSO at −80 °C. Cells were washed and resuspended twice in PBS buffer, quantified using a Scepter Handheld Automated Cell Counter (Millipore, Billerica, MA), and brought up to a working concentration between 200,000 and 10,000,000 cells/mL. 50 μL aliquots of the appropriate dilution of cells were added to swabs which were air dried at room temperature overnight. A reference profile for 1000 F was obtained as described above for buccal swabs. The 1000 M cell line is the same as component F in the National Institute of Standards and Technology (NIST, Gaithersburg, MD) DNA Profiling Standard SRM 2391c and the certified profile from NIST was used as the reference for concordance.

Blood samples in EDTA tubes from three different donors were purchased from Memorial Blood Center (Minneapolis, MN). Two-fold serial dilutions of blood from each donor (20–2.5 μL and 1 μL) were applied to swabs. To prepare these swabs, an aliquot of each blood dilution was pipetted onto a glass slide. Then, a swab wetted with sterile water was used to recover the diluted blood from the slide. The concentration of DNA in each blood sample was determined to calculate the amount being applied onto the swab at each dilution. DNA was extracted from 40 μL of blood from each donor using PrepFiler Forensic DNA Extraction kit (ThermoFisher Scientific) and the amount of DNA quantified in triplicate with Quantifiler Human DNA Quantification Kit (ThermoFisher Scientific) on a Applied Biosystems 7500 Real-Time PCR system v1.4 according to the manufacturer's protocols [17,18].

The DNA Profiling Standard SRM 2391c, produced by NIST (Gaithersburg, MD), was used to test the accuracy of allele calls against NIST certified genotypes. For testing on the RapidHIT System, DNA from components A–D were added to the GlobalFiler Express STR reagents at 1–2 ng/20 μL.

DNA samples from several common animal species (bovine, chicken, horse, porcine, rabbit) were obtained from Biochain (Newark, CA). Purified genomic DNA from several human-associated micro-organisms in the oral cavity was purchased from ATCC (Manassas, VA).

2.2. PCR amplification, sample electrophoresis, and data analysis

2.2.1. 9700 thermal cycling and 3130xL analysis

Buccal swab lysates were prepared to generate a reference database for concordance studies as described above. PCR amplification reactions were prepared by combining 6 μL of GlobalFiler Express primer mix, 6 μL of master mix, and 3 μL of buccal cell lysate to give a total reaction volume of 15 μL according to the manufacturer's protocol [12]. For positive control DNA 007 (supplied in the GlobalFiler Express Kit, ThermoFisher Scientific) reactions, 6 μL of primer mix, 6 μL of master mix, and 1 μL of sterile water was combined and then 2 μL of control DNA 007 (2 ng/μL) was added. Thermal cycling was performed on the GeneAmp® PCR system 9700 (ThermoFisher Scientific) with a 96-well gold-plated silver block. Thermal cycling parameters used the 9700 max mode: enzyme activation at 95 °C for 1 min, followed by 26 cycles of denaturation at 94 °C for 3 s and annealing/extension at 60 °C for 30 s. A final extension step was performed at 60 °C for 8 min, followed by a final hold at 4 °C if the PCR products were to remain in the thermal cycler for an extended time. Cycle number was increased to 27 when re-amplifying samples with partial profiles.

Following thermal cycling, samples were prepared for capillary electrophoresis (CE) according to the manufacturer's protocol with GeneScan™600 LIZ® v2 and 500 LIZ® size standards [12]. Separation was performed on a 16-capillary 3130*xL* Genetic Analyzer (ThermoFisher Scientific) using a 36 cm capillary array, HIDFragmentAnalysis36_POP4 run module with dye set J6. If a sample yielded off-scale peaks it was rerun after decreasing injection parameters from 3 kV for 10 s to 2 kV for 5 s. The electrophoresis results were analyzed using GeneMapper *ID*-X v1.4 genotyping software (ThermoFisher Scientific) using a 20% global filter and the recommended analysis settings for GlobalFiler® Express v1.2 chemistry. Peak amplitude of 50 RFU (relative fluorescence units) was used as the peak detection threshold when analyzing data from all electropherograms.

2.2.2. RapidHIT System

PCR reaction mix for the RapidHIT System was prepared using the same ratios as suggested by the manufacturer [12]. The primer mix and master mix reagents were preloaded into two separate vials prior to insertion of vials onto the sample cartridges. 20 μL of primer mix plus 5 μL of sterile water was combined and added to one vial and 20 μL of master mix plus 5 μL of sterile water was combined and added to the second vial. The two vials were inserted onto the cartridge for each PCR reaction. Once the paramagnetic beads containing extracted, purified DNA were transferred to the PCR reaction chamber, the master mix and primer mix were dispensed simultaneously into the chamber. The total volume of the PCR amplification chamber was approximately 20 μL.

The thermal cycling parameters used were enzyme activation at 96 °C for 1 min, followed by 28 cycles of denaturation at 96 °C for 5 s and annealing/extension at 60 °C for 40 s with a final extension step of 60 °C for 8 min. Upon completion of thermal cycling, all amplified product was transferred to the dilution chamber containing MapMarker® DY632-500 bp size standard (Bioventures). The diluted

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