Evaluation of the Investigator® 26plex QS STR Kit and Comparison with Two Commercially Available STR Kits

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ABSTRACT

Short tandem repeats (STRs) are the gold standard in forensic human identification. Several multiplex STR kits are currently on the market, and in recent years, kits have included an increasing number of loci, resulting in profiles with more discriminatory power. Before new kits are implemented in crime laboratories, they go through extensive validation studies. It is important that the chemistries be sensitive enough to produce profiles from low quantity and/or quality samples. In addition, DNA profiling can be complicated by various PCR inhibitors common to forensic sample types, and some kits are better able to handle these inhibitors than others.

In this study, we evaluated the Investigator® 26plex QS kit (QIAGEN) and compared it to two kits commonly used in forensic laboratories: the Investigator® 24plex QS kit (QIAGEN) and the GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific). The Investigator® 26plex QS kit is a new kit that simultaneously amplifies the CODIS loci and the European standard loci, plus Penta D, Penta E, D6S1043, DYS391, and Amelogenin, along with QS markers to monitor for inhibition. A sensitivity study consisted of varying concentrations of control DNA between 16 pg-2 ng. To test the kits' tolerance to common inhibitors, low, medium, and high concentrations of hematin, humic acid, calcium, and collagen were added to control DNA. In addition, a study was carried out to assess the effect of male/female DNA mixtures on profile interpretation. Finally, a variety of casework-type samples were run, including bone, hair, blood, decomposed muscle, UV-damaged, buried bloodstains, and formalin-damaged tissue. Overall, this research investigates and reports on the relative performance of three commercial STR chemistries, including a chemistry not currently available in the United States.

INTRODUCTION

Environmental factors such as UV exposure, heat, humidity, and presence of microorganisms can result in DNA damage and degradation (1). This may affect DNA profiling by causing allele or locus dropout, low peak heights, and peak height imbalance. Additionally, DNA profiling can be complicated by various PCR inhibitors being co-extracted with DNA (2). Inhibitors such as hematin, collagen, calcium, and melanin are naturally present in biological samples. Others, including humic acid, may be introduced from the environment, such as when remains are buried. Inhibitors may cause complete PCR failure or reduce PCR efficiency, resulting in dropout of some alleles, lower peak heights, and reduced peak height ratios (2).

The Investigator® 24plex QS and Investigator® 26plex QS kits include two quality sensors (QS1 and QS2), which act as internal PCR controls to confirm amplification and monitor for inhibition (3,4). QS1 and QS2 are 74 bp and 435 bp amplicons, respectively, and inhibition causes the larger amplicon to amplify less efficiently or to drop out entirely. It is recommended that a 20% QS2/QS1 ratio be used for determining whether inhibition has occurred (manufacturer recommendation).

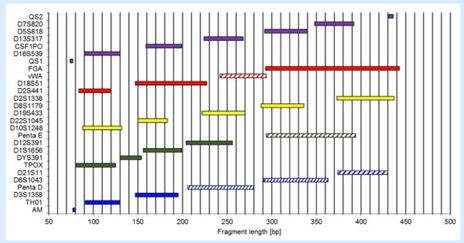

Figure 1. Layout of Loci for the Investigator® 26plex QS Kit.

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RESULTS & DISCUSSION

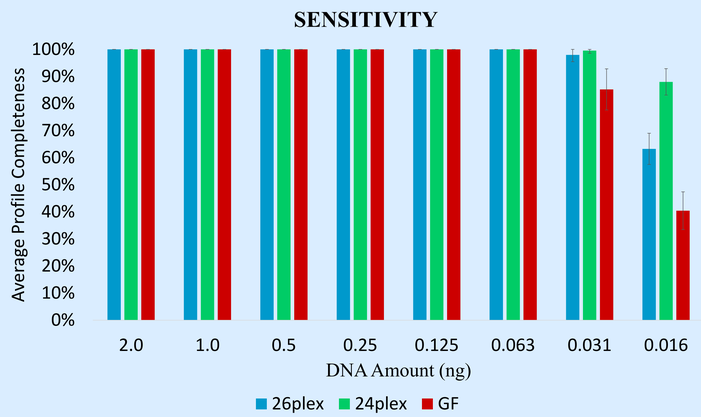

Figure 2. Sensitivity of STR Kits. DNA amounts of 16 pg to 2 ng were amplified with five replicates for each kit. Data represent the average percentage of expected alleles seen ± the standard deviation.

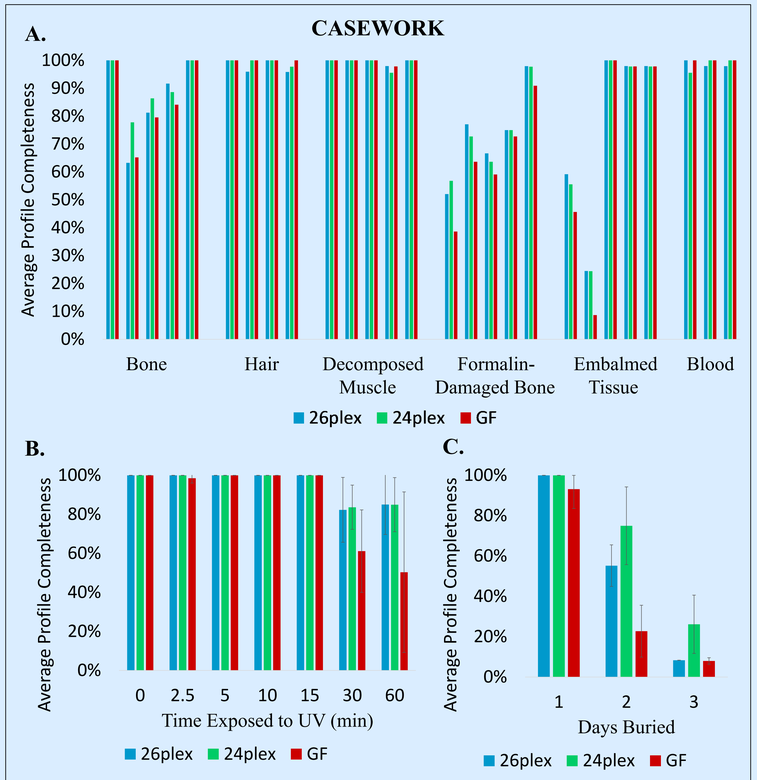

Figure 4. Casework-type Samples. Data represent the average percentage of expected alleles seen ± the standard deviation for various casework-type samples, including bone, hair, decomposed muscle, formalin-damaged bone, embalmed tissues, and blood on filter paper (A; no replicates); blood on filter paper exposed to UV (B; average of three samples), and buried blood swabs (C; average of two replicates). Note: More alleles expected for Investigator® 26plex QS.

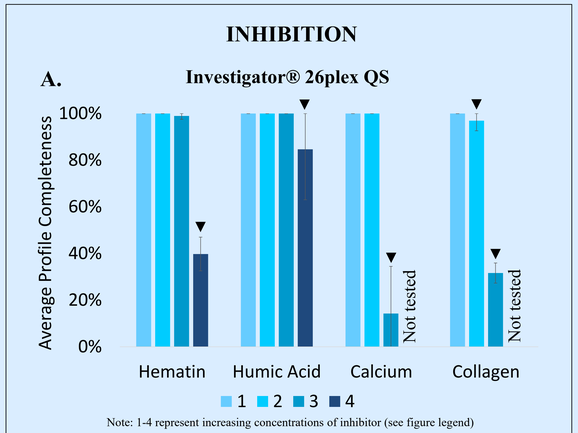

Figure 3. Inhibition of STR Kits. Control DNA was spiked with common PCR inhibitors and run in duplicate with Investigator® 26plex QS (A), Investigator® 24plex QS (B), and GlobalFiler (C). Data represent the average percent of expected alleles ± standard deviation. Inhibitor concentrations tested were as follows:
Hematin (pM): 1 = 250; 2 = 500; 3 = 1000; 4 = 4000
Humic Acid (ng/µL): 1 = 50; 2 = 150; 3 = 300; 4 = 1200
Calcium (mM): 1 = 1; 2 = 3; 3 = 5; 4 = 20
Collagen (ng/µL): 1 = 100; 2 = 200; 3 = 300; 4 = 800
▼ indicates S/Q ratio below 20% for one or both replicates

MATERIALS & METHODS

SENSITIVITY

Taqman® Control Human Genomic DNA (Thermo Fisher Scientific) at 2 ng, 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng, 0.03125 ng, and 0.015625 ng; 5 replicates each. Additionally, DNA amounts of 3 and 5 ng were tested with only the Investigator® 26plex QS kit

INHIBITION

Taqman® Control Human Genomic DNA (Thermo Fisher Scientific); 0.5 ng DNA amplified spiked with different concentrations of hematin, humic acid, calcium, or collagen; 2 replicates each

MIXTURES

Reference buccal swabs from a male and female extracted using the EZ1 DNA Investigator Kit (QIAGEN) and normalized. Mixed in ratios of 1:1, 1:3, 1:10, 1:15, 3:1, 10:1, and 15:1 male DNA to female DNA. DNA input amounts of 0.8 ng and 1.5 ng, 2 replicates each

CASEWORK-TYPE SAMPLES

Bones (N=5); rooted hairs (N=4); muscle tissues of decomposing cadavers (N=5); formalin-damaged bones (N=5) exposed for 0, 0.25, 14, 24, and 66 hours; embalmed tissues (N=5); blood on filter paper (N=3); UV-damaged bloodspots (N=21) exposed for 0, 2.5, 5, 10, 15, 30, and 60 minutes in a UV cross-linker; blood swabs (N=6) buried in soil for 1-3 days

DNA EXTRACTION

EZ1 DNA Investigator Kit (QIAGEN) – buccal swabs, rooted hairs, blood on filter paper, UV-damaged bloodspots, and buried blood swabs
Prepfiler® BTA Forensic DNA Extraction Kit – bones, formalin-damaged bones
QIAamp® DNA Investigator Kit – decomposing muscle tissue
QIAamp® DNA FFPE Tissue Kit (QIAGEN) – embalmed tissues

DNA QUANTIFICATION

Investigator Quantiplex Pro Kit (QIAGEN)

STR AMPLIFICATION

Investigator® 26plex QS PCR Amplification Kit (QIAGEN)
Investigator® 24plex QS PCR Amplification Kit (QIAGEN)
GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific)

CAPILLARY ELECTROPHORESIS AND DATA ANALYSIS

3500 Genetic Analyzer (Thermo Fisher Scientific)
GeneMapper® ID-X Software v1.4 (Thermo Fisher Scientific)

CONCLUSIONS

- All three kits are sensitive to 63 pg of DNA; at an input of 16 pg, 24plex produced the most complete profiles (Figure 2)
- 26plex has the potential to handle higher inputs of DNA than the other kits with fewer artifacts (data not shown)
- High concentrations of calcium and collagen had the most inhibitory effect on 26plex and GlobalFiler; 24plex showed the highest tolerance to all inhibitors (Figure 3)
- All three kits resulted in comparable profile completeness for casework-type samples (Figure 4)
- Alleles at loci shared between the kits were concordant for all samples tested
- Full (or nearly full) profiles for major and minor contributors were recovered from 1:1, 3:1, and 10:1 mixtures; dropout occurred for the minor contributor in 15:1 mixtures (data not shown)

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