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Research paper

Developmental validation of the DNAscan™ Rapid DNA Analysis™ instrument and expert system for reference sample processing

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ABSTRACT

Since the implementation of forensic DNA typing in labs more than 20 years ago, the analysis procedures and data interpretation have always been conducted in a laboratory by highly trained and qualified scientific personnel. Rapid DNA technology has the potential to expand testing capabilities within forensic laboratories and to allow forensic STR analysis to be performed outside the physical boundaries of the traditional laboratory.

The developmental validation of the DNAscan/ANDE Rapid DNA Analysis System was completed using a BioChipSet™ Cassette consumable designed for high DNA content samples, such as single source buccal swabs. A total of eight laboratories participated in the testing which totaled over 2300 swabs, and included nearly 1400 unique individuals. The goal of this extensive study was to obtain, document, analyze, and assess DNAscan and its internal Expert System to reliably genotype reference samples in a manner compliant with the FBI's Quality Assurance Standards (QAS) and the NDIS Operational Procedures.

The DNAscan System provided high quality, concordant results for reference buccal swabs, including automated data analysis with an integrated Expert System. Seven external laboratories and NetBio, the developer of the technology, participated in the validation testing demonstrating the reproducibility and reliability of the system and its successful use in a variety of settings by numerous operators. The DNAscan System demonstrated limited cross reactivity with other species, was resilient in the presence of numerous inhibitors, and provided reproducible results for both buccal and purified DNA samples with sensitivity at a level appropriate for buccal swabs. The precision and resolution of the system met industry standards for detection of micro-variants and displayed single base resolution. PCR-based studies provided confidence that the system was robust and that the amplification reaction had been optimized to provide high quality results.

The DNAscan integrated Expert System was examined as part of the Developmental Validation and successfully interpreted the over 2000 samples tested with over 99.998% concordant alleles. The system appropriately flagged samples for human review and failed both mixed samples and samples with insufficient genetic information. These results demonstrated the integrated Expert System makes correct allele calls without human intervention.

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1. Introduction

The generation of short tandem repeat (STR) profiles for forensic purposes and upload to CODIS is currently conducted within accredited laboratory facilities. The processes require qualified forensic scientists and specialized instrumentation, all housed within a clean, controlled workspace. Over time, the usefulness of forensic DNA testing has become evident for criminal cases and arrestee testing and a need has developed for a DNA analysis solution that is fast, portable, and operable by non-technical personnel. "Rapid DNA" is defined by the Federal Bureau of Investigation (FBI) as follows: "Rapid DNA describes the fully automated (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab. The 'swab in – profile out' process consists of automated extraction, amplification, separation, detection and allele calling without human intervention" [1]. A consortium of U.S. government agencies including the FBI, Department of Defense, and Department of Homeland Security addressed their critical needs for Rapid DNA technology by establishing the Accelerated Nuclear DNA Equipment (ANDE) program. The DNAscan/ANDE Rapid DNA Analysis System was developed as a result of that program. The DNAscan system is also sold as the ANDE™ (Accelerated Nuclear DNA Equipment) Rapid DNA Analysis System. Both systems perform identical sample processing and the ANDE system has additional features including enhanced ruggedization. In this work, both systems will be referred to as DNAscan.

NDIS approval of the fully integrated DNAscan System, including Expert System data interpretation without human intervention, will allow Rapid DNA STR profile generation and searching of the CODIS database for the first time. Ultimately and with requisite legislative changes, rapid STR profile generation may also be performed outside the laboratory in general and in the police station in particular. These fundamental shifts have the potential to dramatically improve societal safety by revolutionizing the speed and manner in which suspects are identified, enrolled in the national criminal history system and the CODIS database, and searched against unsolved criminal cases. As additional countries adopt Rapid DNA technology, similar policy changes may increase safety and security worldwide.

1.1. Overview of the DNAscan rapid DNA analysis system

The DNAscan System consists of three primary components: the BioChipSet Swab, the BioChipSet Cassette, and the DNAscan instrument. Up to five swabs are simply inserted into the cassette and the cassette loaded into the DNAscan instrument [Fig. 1]. The instrument door is then closed and the run initiates automatically.

The BioChipSet Cassette is a single use, room temperature stable, disposable consumable which includes all reagents, materials and waste containment required to perform STR analysis [2]. The DNA purification reagents, STR reagents, buffers, and separation polymer are all pre-loaded on the cassette and have been optimized for the microfluidic environment to ensure consistent, balanced, and precise results. Electrophoretic separation channels are injection molded into the single use cassette.

The DNAscan instrument can be placed on a standard tabletop and can easily be moved by two individuals using the carry handles on either side of the instrument. The instrument has passed U.S. Military Standard 810F for shock and vibration, which signifies that each corner of the instrument can withstand a drop from 4 inches outside the carrying case and the vibration expected during transportation by plane, truck, and hand carry. After being moved, the DNAscan instrument requires no manual calibration.

The DNAscan instrument is comprised of several subsystems, including a pneumatic subsystem for driving fluids throughout the cassette, a thermal subsystem for performing multiplexed amplification, a high voltage subsystem for electrophoresis, an optical subsystem for exciting and detecting fluorescently labeled STR fragments during electrophoresis, and a ruggedization subsystem to allow transport and field forward operation without recalibration or optical realignment [2]. The instrument's single board computer controls subsystem functions, performs data processing, interfaces with the user through an integrated touch screen, and provides ethernet and USB connectivity.

Fig. 1. The BioChipSet Cassette can be loaded into the DNAscan instrument by non-technical personnel.

To complement the instrument and cassette design, the DNAscan System includes several integrated software packages for instrument control, data collection, quality control, and Expert System STR analysis of sample files [Fig. 2].

The Expert System software automatically analyzes the data after run completion and provides rapid feedback on the usability of the STR profiles for database searching. A green checkmark, yellow checkmark recommending analyst review, or red "X" on the DNAscan touch screen indicates whether the result of each lane is successful. In all cases, the output files are available for review by a forensic scientist if desired. All output files are encrypted and can be exported and decrypted based on the multi-level user privilege design.

1.2. Developmental validation approach

The developmental validation was performed to demonstrate reliability, reproducibility and robustness of the DNAscan Rapid DNA Analysis System, including the integrated Expert System, across a number of laboratories and buccal sample variations. The practical knowledge and subject matter expertise of accredited, NDIS-participating laboratories was sought and integrated into the design and execution of the experiments. This expertise was critical to analyze and assess if the data generated by DNAscan and its internal Expert System reliably genotyped reference samples in a manner compliant with the FBI's Quality Assurance Standards (QAS) and the NDIS Operational Procedures.

The design and execution of a developmental validation for Rapid DNA technology required application of validation guidelines in a new manner. Developmental validations commonly push the limits of the system to aid laboratories in setting the specific conditions to be used in their laboratories such as PCR cycle numbers. By design, the DNAscan System does not allow user intervention or manipulation of the performance parameters during analysis or data interpretation and the validation approach reflects that difference. Additionally, the DNAscan System

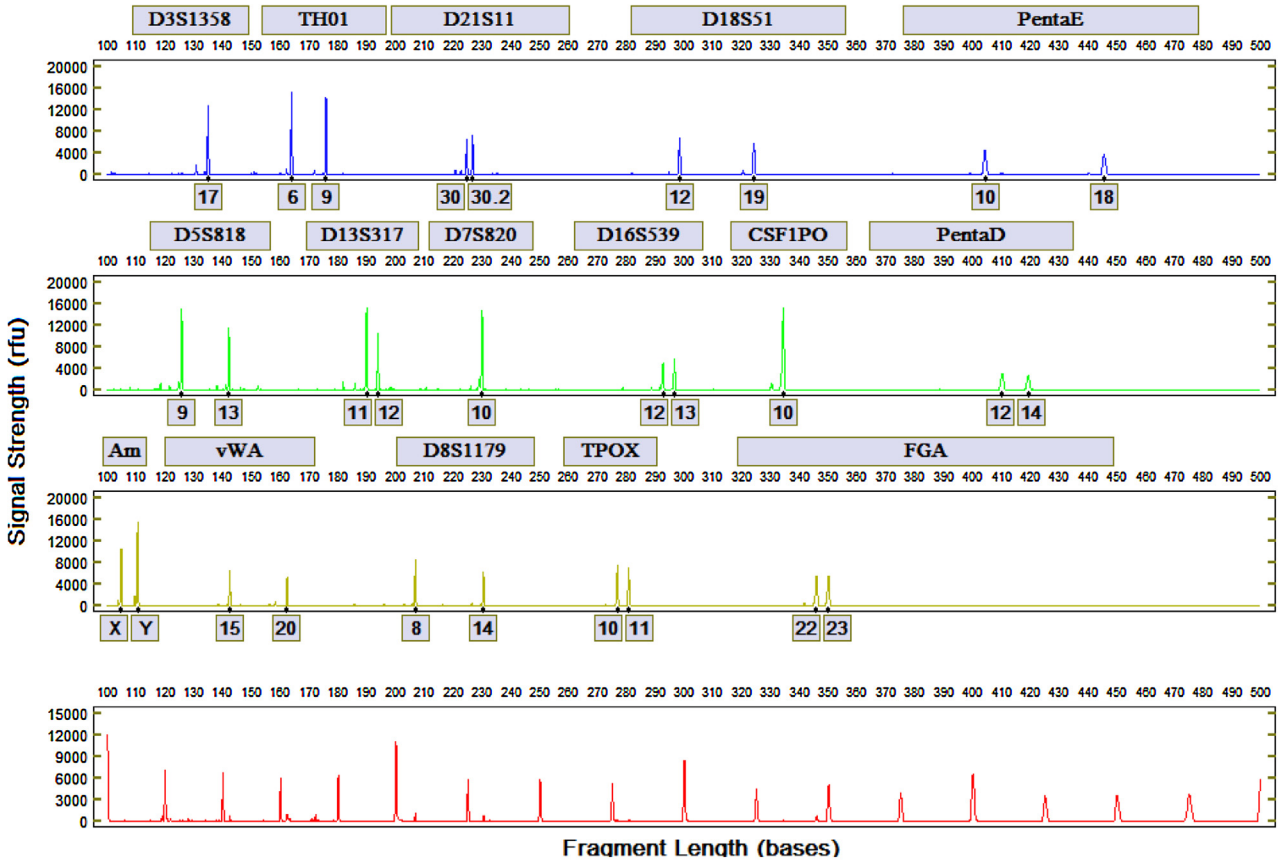

Fig. 2. Representative electropherogram developed by the DNAscan Expert System.

integrates both the amplification chemistry and the Expert System which have traditionally been validated separately. Therefore, the approach used for this developmental validation was aimed at striking a balance between performing almost all of the studies commonly performed while taking into account the simplicity of the "swab-in, profile out" Rapid DNA technology [3,4].

The amplification chemistry validated on the DNAscan System uses the Powerplex 16 System (Promega Corporation, Madison, WI) primer sequences and includes the 13 CODIS core loci plus amelogenin, Penta D, and Penta E [5,6]. The multiplex STR amplification reaction has been optimized for a rapid microfluidic environment [7] and is performed using a 7 μL reaction with 31 cycles being completed in 19.7 min. While the Powerplex 16 System (Promega Corporation) was approved by NDIS over 10 years ago, the optimization and lyophilization of the amplification chemistry, combined with microfluidic engineering on the cassette for use on a Rapid DNA instrument, warrant a full developmental validation.

The DNAscan System employs a fully automated, fully integrated allele calling and Expert System software package, allowing it to satisfy the FBI's definition for Rapid DNA (as opposed to "Modified Rapid DNA," which requires human interpretation and technical review) [1]. The Expert System was designed and specifically tailored for the PowerPlex 16 data generated by the DNAscan System. All data generated during the developmental validation was automatically processed by the integrated Expert System such that the fully integrated DNAscan System can be used to generate and interpret single source buccal samples without human intervention.

A final consideration in the design of the DNAscan System developmental validation was the choice of sample type for each study. Traditional studies are typically conducted using controlled, laboratory-developed samples to ensure the samples contain a specified amount of DNA to evaluate the limits of the new technology. This approach allows the samples to remain constant while the parameters, reagent concentrations, and other configurable settings are varied. The approach used in validating DNAscan System was very different because the system is intended for use with single source buccal samples and, by design, there are essentially no user-configurable settings. Therefore, "real-world" samples in the form of buccal samples collected from random individuals were used in the vast majority of developmental validation testing, with the major exception being the study of species specificity.

2. Materials and methods

In addition to NetBio, the developer of the DNAscan System, the seven external laboratories participating in the developmental validation were the Alabama Department of Forensic Sciences, Michigan State Police Forensic Science Division, Florida Department of Law Enforcement DNA Investigative Support Database, Pennsylvania State Police Bureau of Forensic Services, Dubai Police GHQ, Gen. Dept. Forensic Sciences & Criminology, Defense Forensic Science Center, and National Institute of Standards and Technology. Each external laboratory was provided a unique DNAscan System for performing the requested testing with a total of 14 DNAscan instruments used in the study. All DNAscan Systems were builds of the same product code with identical versions of software. The

study was approved by an Institutional Review Board; all buccal swab donors reviewed a Research Subject Information Sheet and provided informed consent.

Samples were tested using the DNAscan System as described in accordance with manufacturer's recommendations. Briefly, buccal swabs were collected on NetBio swabs and those swabs were placed into the BioChipSet Cassette. The BioChipSet Cassette was then placed into the DNAscan instrument for fully-automated sample processing, allele calling, and data interpretation with the integrated DNAscan Expert System.

2.1. Species specificity

Testing of a total of nineteen (19) different species, fourteen (14) animal and five (5) types of bacteria, was performed in duplicate using a similar quantity of DNA as typically collected on a human buccal swab, 1000 nanograms [2]. For each of the following, chimpanzee, gorilla, orangutan, macaque, cow, dog, rat, cat, mouse, rabbit, horse, chicken, pig, and ferret, 1000 nanograms of genomic DNA in 50 μL of TE^{-4} were placed on individual NetBio swabs. Genomic DNA from *Streptococcus pneumoniae* (4.89×10^7 genome equivalents), *Staphylococcus aureaus* (3.49×10^7 genome equivalents), *Lactobacillus plantarum* (2.92×10^7 genome equivalents), *Escherichia coli* (2.13×10^7 genome equivalents), and *Candida albicans* (6.3×10^6 genome equivalents) in 50 μL of TE^{-4} were placed on individual NetBio swabs. Primate genomic DNAs were obtained from the Coriell Institute for Medical Research (Camden, NJ), and the other mammalian genomic DNAs were obtained from Zyagen (San Diego, CA). Bacterial and fungal genomic DNAs were purchased from ATCC (Manassas, VA).

2.2. Sensitivity

Four laboratories tested buccal swabs collected from 5 unique individuals using 1 swipe, 3 swipes, and 6 swipes as described in the Instructions for Buccal Cell Collection using the DNAscan Collection kit [8]. The recommended number of swipes for sample collection is six (6), with one up-and-down motion considered to be a single swipe.

Purified DNA samples were created by processing the entire tip of one swab using a guanadinium-based extraction [9] which is essentially a tube-based version of the DNAscan instrument protocol, and quantified using a Nanodrop™ 2000C Spectrophotometer (ThermoFisher Scientific, Waltham, MA). Purified DNA from the swabs was pooled to produce a stock DNA solution. The stock DNA solution was diluted in TE^{-4}, pH 8 buffer to prepare 500 μL solutions of 40, 20, 10, 5 and 2 ng/μL. Swab samples were then spiked by pipetting 50 μL of diluted DNA solution onto the swab head.

2.3. Stability

Buccal swabs were collected from two unique donors on NetBio swabs and stored in their protective clear plastic tube for 0 (tested immediately), 1, 2, or 7 days in environmental chambers at both 22 °C (room temperature) and 4 °C. Swabs were collected and immediately placed in the plastic storage tube without air drying.

2.4. Inhibitors

The following twenty (20) potentially inhibitory substances that are likely to be found in the oral cavity were tested: beer, bloody swab, Cheetos®, chocolate, cigarette, coffee (brand 1), coffee (brand 2), gum (brand 1), gum (brand 2), lip balm, lipstick, mint, mouthwash, soda (brand 1), soda (brand 2), sugar, tea, tobacco dip, toothpaste (brand 1), toothpaste (brand 2). The potential inhibitors were consumed by the donor in a manner similar to reasonable use prior to buccal swab collection. For example, gum was chewed for approximately 5 min just before standard buccal swab collection was performed. The only exceptions were the "bloody swab" created by depositing a drop of blood from a finger stick onto the swab and the "coffee" swab created by pipetting 100 μL of coffee directly onto the previously collected buccal swab. Each substance was tested in duplicate. Wherever possible the potential inhibitors were consumed by the donor in a manner similar to reasonable use prior to buccal swab collection.

2.5. Reproducibility

All eight (8) laboratories were provided the same ten (10) unique buccal samples in duplicate, and tested them using two different lots of BioChipSet cassettes.

2.6. Mixtures

Buccal swabs were collected from 2 donors and the DNA was extracted by processing the entire tip of one swab using a guanadinium-based extraction [9] which is essentially a tube-based version of the DNAscan instrument protocol, and quantified using a Nanodrop 2000C Spectrophotometer (ThermoFisher Scientific). Once quantified, the purified DNA was mixed in the ratios of 19:1, 5:1, 1:1, 1:5, and 1:19 yielding a total of two micrograms (2 μg) of DNA in 50 μL. The mixed DNA was deposited on individual swabs for each ratio.

2.7. Accuracy and concordance

Several buccal swabs were collected from each donor, 1398 unique donors being tested for accuracy and concordance during the validation, following the standard protocol for buccal cell collection from the inside of the cheek using the NetBio swab [8]. The moist swab was then placed in the plastic storage tube. The swabs were stored at room temperature immediately after collection and then at approximately 4 °C before being shipped to the laboratories for testing.

The samples included in the accuracy set were used to evaluate concordance to conventional laboratory testing, signal strength, success rate, and resolution. The samples were also used in the contamination studies.

2.8. Conventional laboratory testing

One replicate of the swab collection from each donor from the following studies was sent for conventional laboratory STR testing at The Bode Technology Group, Inc.: Accuracy, Inhibitors, Sensitivity, Stability, and Reproducibility. During the conventional laboratory testing, DNA was extracted from the buccal swabs using the BioSprint 96 Robotic Workstation (Qiagen, Hilden Germany) and BioSprint 96 DNA Blood Kit (Qiagen). Approximately 0.5–2.0 ng of the extracted DNA was amplified using the PowerPlex 16 HS System (Promega Corporation) and detected using an Applied Biosystems® 3130 Genetic Analyzer (ThermoFisher Scientific). The data was analyzed using Genemapper® ID-X, version 1.1 (ThermoFisher Scientific) and interpreted by two qualified analysts. Additional validated techniques such as re-injection, re-extraction, quantification, and increased cycle numbers were employed for samples that did not initially pass technical specifications for reporting.

3. Results and discussion

3.1. Species specificity

The species specificity of STR typing systems is essential to ensure that interpretation of STR profiles derived from human subjects is not complicated by other species' cross-reactivity with the assay under study. It is also an important measure to evaluate non-specific binding occurring during amplification that may cause artifacts and anomalies that could complicate data interpretation of single source reference samples. The DNAscan System is intended for human single source buccal samples making microorganisms prevalent in the oral cavity those most relevant for evaluation of cross-reactivity. Samples from other macroorganisms, including primates and domestic animals, were also evaluated.

Of the 19 different species tested, none yielded passing profiles from the DNAscan Expert System. No amplification peaks were called for rabbit, chicken, cow, horse, mouse, cat, dog, gorilla, *Streptococcus pneumonia, Lactobacillus plantarum, Staphylococcus aureas, Escherichia coli,* and *Candida albicans* Several low level peaks were assigned allele calls in the samples for rat, pig, ferret, and macaque. As expected, several called peaks were also detected for the primate samples. All alleles detected and/or called for the species samples were indicated in red warning boxes, indicating a failure to pass one or more of the interpretation rules within the Expert System.

3.2. Sensitivity

Buccal samples are inherently variable in their DNA content, yet typically contain much more DNA than is required for standard PCR amplifications for forensic STR typing. Previously, NetBio has demonstrated that buccal swabs contain a mean of 1267 ng genomic DNA, with an approximately 15-fold range in the amount of DNA template (305–4455 ng/swab), again showing the high variability inherent in buccal samples collected for DNA testing [2]. A typical amplification reaction in the DNAscan instrument targets approximately 1–2 ng of genomic DNA, almost three orders of magnitude less than that contained in a buccal swab. The DNAscan System accomplishes this three order of magnitude reduction and compresses the 15-fold range of DNA product by: 1) subjecting only about 25% of the buccal cell lysate to purification; 2) utilizing a silica matrix that inefficiently binds DNA in general and binds DNA less efficiently at greater DNA concentrations; and 3) diluting the purified DNA prior to the PCR amplification process.

The STR profiles were examined for the presence of expected alleles, signal strength, and peak height ratio. The peak height ratio for each locus was determined for each set of samples that were collected with 6 swipes, 3 swipes, and 1 swipe. All samples that had all thirteen CODIS loci were included in the analysis [Fig. 3]. The average peak height ratio across all loci was 83% for each swipe number, demonstrating excellent intra-locus balance for all heterozygous loci across all dye channels.

The entire buccal swipe swab test set comprised of 120 samples, and, with the exception of 4 swabs, yielded full profiles for the CODIS core loci. Three of the 4 swabs completely failed with no data being passed by the Expert System, and the fourth swab yielded a partial profile with 12 loci.

Additionally, four laboratories tested purified human genomic DNA at 2.0 μg, 1.0 μg, 0.5 μg, 0.25 μg and 0.1 μg quantities. These quantities were selected to bracket the typical amount of DNA expected to be recovered on a buccal swab sample and evaluate the system's performance at the upper and lower DNA levels. The downstream STR profiles were examined for the presence of expected alleles, signal strength, and peak height ratio. The average peak height ratio across all heterozygous loci was 83%, 80%, and 73% for the 2.0 μg, 1.0 μg, and 0.5 μg samples, respectively.

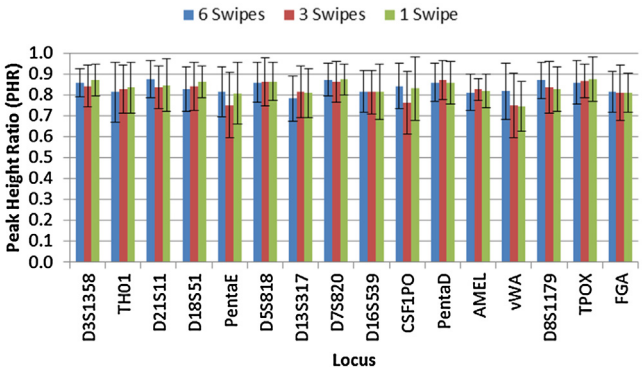

Fig. 3. Peak height ratio with standard deviation for buccal samples yielding a full profile for the CODIS core loci.

The DNAscan system was designed to yield a full profile when 1.0 μg or greater DNA template is present on a buccal swab. While some signal intensity variation was observed across loci, the results of this testing confirm that the system works as originally designed with all samples containing 1.0 μg or greater yielding complete STR profiles. Lower concentrations showed variable results, as expected, with partial profiles detected at concentrations of 0.5 μg and lower. A second DNAscan BioChipSet has been developed to allow Rapid DNA Analysis of forensic samples with lower amounts of input DNA [12].

3.3. Stability

Stability testing using swabs from two donors, was performed to examine the possible effects of short term storage of NetBio swab samples on performance within the DNAscan instrument.

The buccal swabs from donor 1 yielded full profiles at each time points (1, 2, and 7 days) for both room temperature and 4 °C. Imbalanced alleles at Penta D were present on the donor 2 swab stored at 4 °C for one day. Taken together, these results show that the NetBio BioChipSet swabs can be reliably stored at 4 °C for at least 7 days, or at room temperature for up to 7 days, and produce successful typing results.

All reagents have previously been shown to be stable for at least 6 months at 22–30 °C in environmental chambers [2].

3.4. Inhibitors

In a forensic setting, a wide variety of potential inhibitors are often encountered on typical buccal samples collected for DNA analysis and may make it challenging for the laboratory to develop a full STR profile from a reference buccal swab. Although it is recommended that each donor refrain from food and beverage consumption for at least 30 min prior to collection, in practice, standard field-use collection involves buccal swab collections containing potentially inhibitory substances that still reside within the oral cavity at the time of collection.

As a result of this comprehensive study, none of the twenty potential inhibitors impacted the data quality generated on the DNAscan System. Full profiles were generated from swabs containing each of the inhibitory substance, demonstrating that the DNAscan System successfully generates STR profiles when sample collection occurs in the presence of these potential oral inhibitors.

3.5. Reproducibility

Reproducibility of single source reference samples collected and tested on the DNAscan instrument was evaluated within a laboratory, between external participating laboratories, and across multiple lots of cassettes.

Full STR profiles for the CODIS core loci with the expected genotypes were obtained from at least one replicate of each unique donor swab at all of the eight (8) laboratory testing sites, with no discordant types observed [Fig. 4]. Four instances of allele drop-out in non-CODIS core loci were observed; however, it is important to note that the drop-out instances were properly identified by the Expert System.

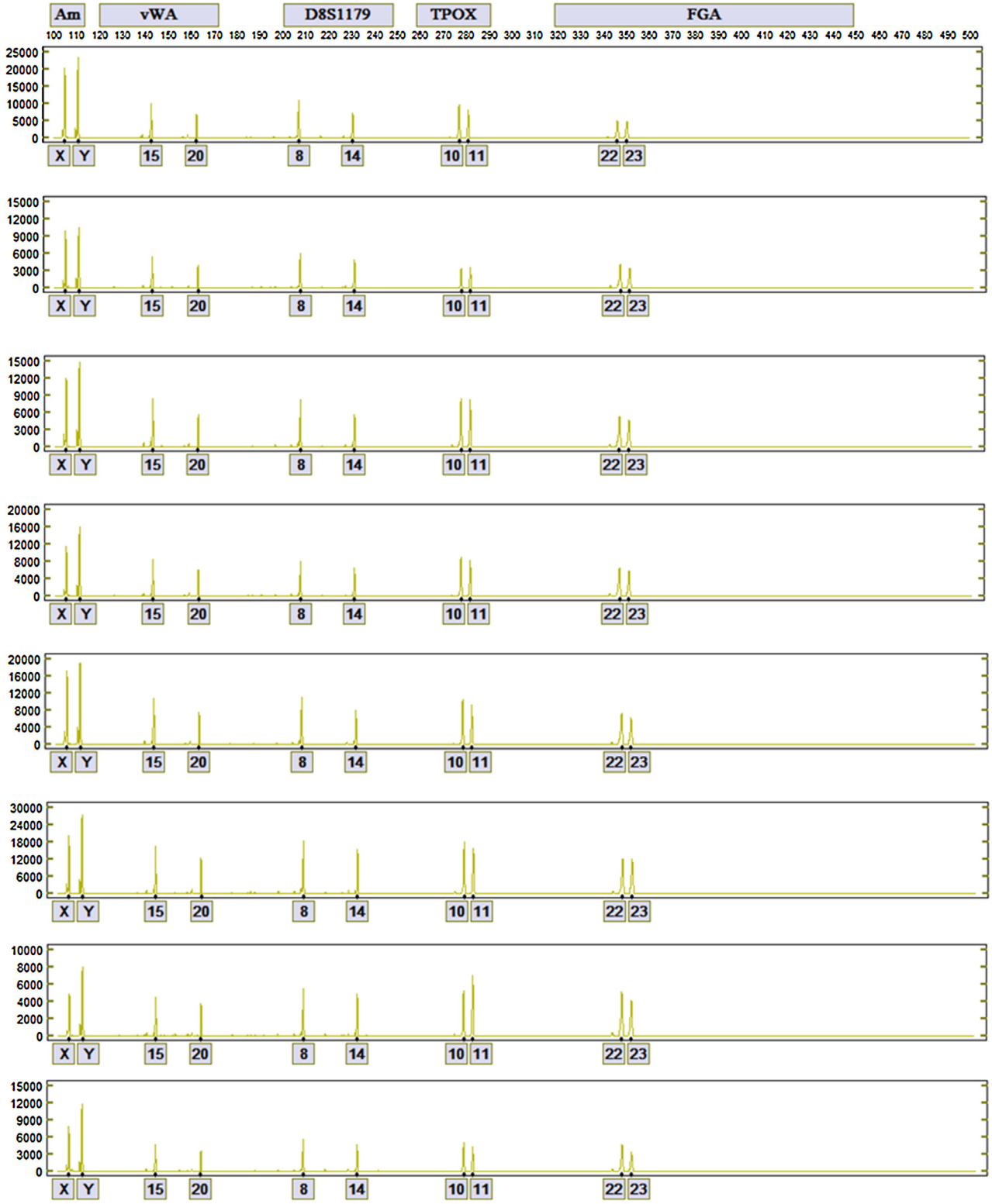

Fig. 4. Representative set from a reproducibility buccal sample for all eight laboratories. Data for the carboxy-tetramethylrhodamine (TMR) channel is shown.

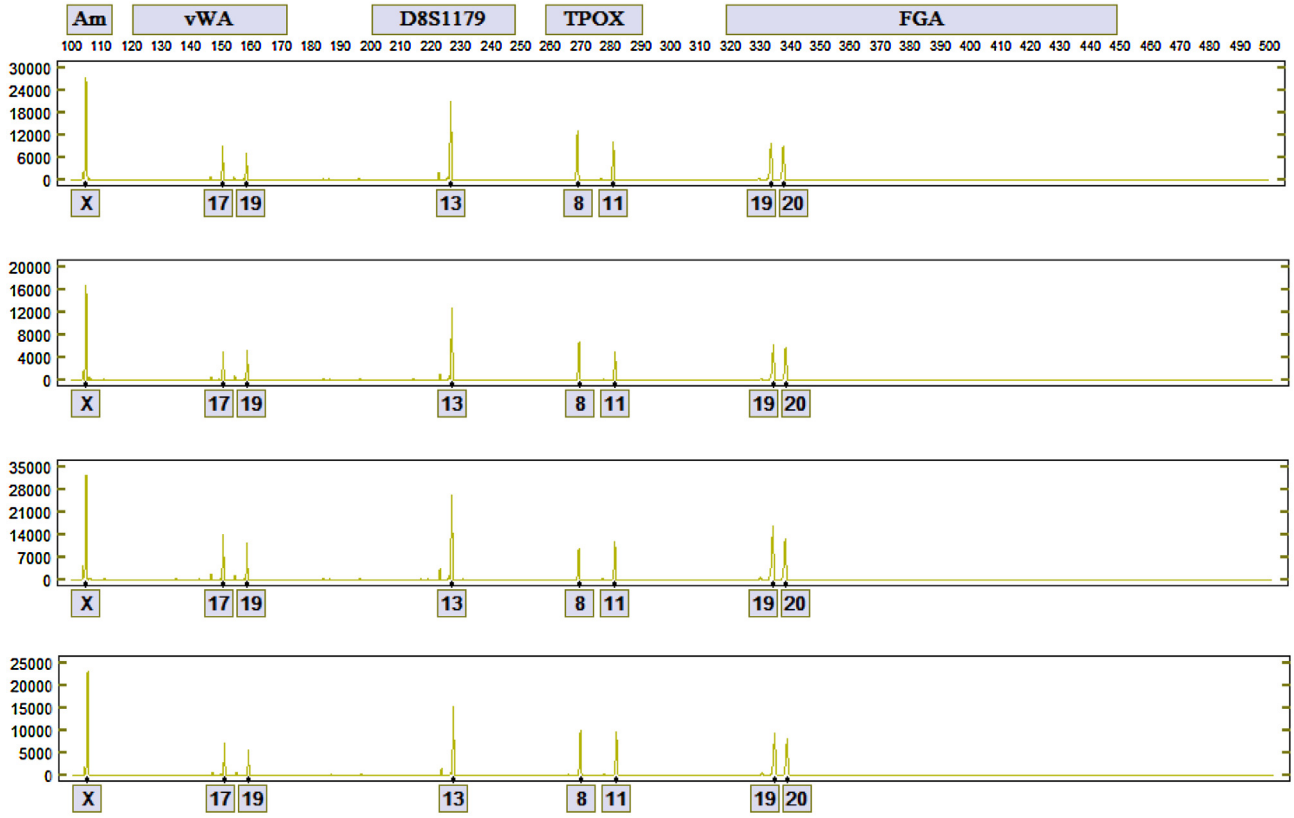

Fig. 5. Representative set from a reproducibility purified DNA sample for four laboratories. Data for the carboxy-tetramethylrhodamine (TMR) channel is shown.

A second Reproducibility Study was performed utilizing extracted and purified DNA in order to evaluate sample reproducibility for purified DNA samples as well as to examine reproducibility without the variable of buccal collection. Four of the laboratories analyzed three unique purified DNAs in duplicate using two different lots of cassettes. Each of the laboratory sites testing the purified DNA swabs obtained full and concordant profiles for all of the purified DNA samples [Fig. 5].

Reproducibility of the DNAscan System was also evaluated by generating results from a NIST-traceable swab. Although evaluation of a NIST Standard Reference Material (SRM; or a standard traceable to a NIST standard) is not a specific requirement for developmental validation, the FBI's Quality Assurance Standard 9.5.5 requires SRM testing whenever substantial changes are made to a procedure [14]. Accordingly, the evaluation was performed during developmental validation. The commercially available NIST SRM 2391c (PCR-Based DNA Profiling Standard) commonly used for validation of forensic DNA applications contains four Components (A–D) of genomic DNA in TE^{-4} buffer [10] and the total amount of DNA contained within these components is approximately 55–105 ng. Since the DNAscan System was optimized for a target input of 1000 ng (1 μg) of DNA, Components A-D are not suitable and therefore were not tested on the DNAscan System due to their insufficient quantity of DNA template. Instead, a NetBio swab was run in parallel with SRM 2391c Components A-C thereby creating a NIST-traceable swab. Next, five replicates of the NIST SRM 2391c traceable swabs were run in each of the five lanes of a single cassette and the data was analyzed using DNAscan with integrated Expert System. All of the samples yielded full and concordant profiles.

3.6. Mixtures

Although mixtures are not commonly encountered in the testing of reference samples, it was important to assess whether the DNAscan System with its integrated Expert System would effectively detect and interpret mixed STR profiles in the rare case a true mixed DNA sample was collected, or in the event contamination of the associated buccal swab occurred.

In all cases, STR profiles were reliably detected and appropriately failed by the Expert System for all mixed DNA samples detected using the DNAscan instrument with the integrated Expert System.

3.7. Precision and accuracy

3.7.1. Precision

Inter-run precision was calculated for all fourteen instruments used in this study and was determined based on allelic ladders from 418 runs. The standard deviation in bases was calculated for each allele in the allelic ladder [Fig. 6] and ranged from 0.006 bases at D8S1179 to 0.071 bases at Penta D 17. The variation at three standard deviations range from 0.018 bases to 0.213 bases, and are well below the acceptable target value of 0.5 bases.

3.7.2. Concordance

A total of 1368 samples were assessed for concordance of the CODIS core 13 loci. Only passing samples with alleles reported to the DNAscan allele table and .xml file were included in the concordance calculations. Concordance was evaluated by comparing profiles from the same donor between the DNAscan System and the corresponding STR profiles generated using conventional

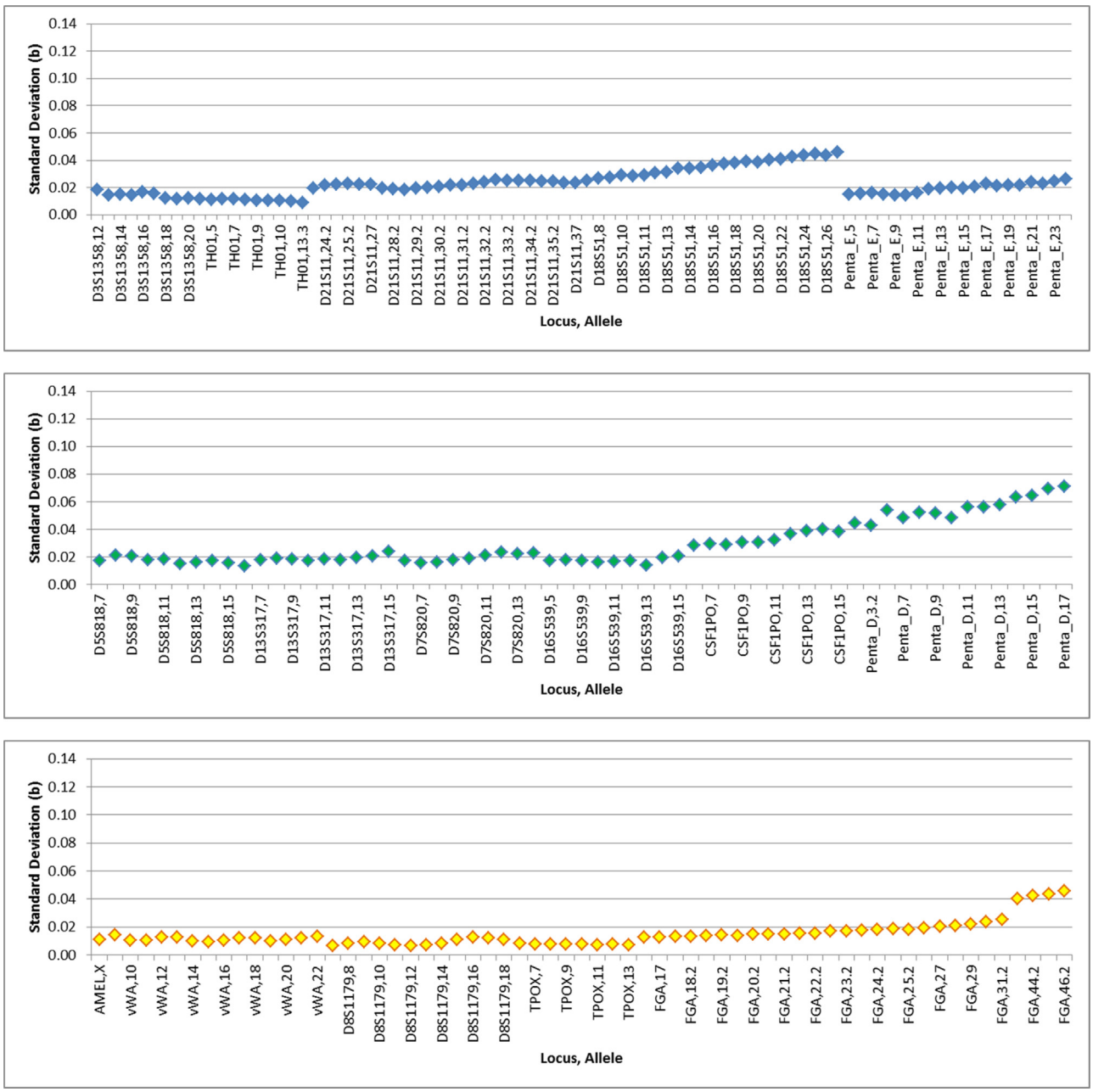

Fig. 6. Sizing variation at a single standard deviation for each allele in the allelic ladder calculated for 418 runs on 14 DNAscan instruments.

STR typing and the PowerPlex 16 HS System (Promega Corporation) performed at the Bode Technology Group, Inc.

The accuracy allele calling rate was 99.998% with only one single allele being discordant due to allele drop-out at D7S820. The DNAscan correctly detected the allele calls of 10, 11; however, the 11 allele did not meet the peak height ratio threshold requirement of the Expert System and therefore was not designated.

3.7.3. Signal strength

The signal strength for each locus was calculated for each of the 1362 donor samples by summing the signal strengths of all called peaks within the locus and dividing by two. The average peak height ranged from 2294 relative fluorescence units (rfu) at Penta D to 9089 rfu at D13S317 [Fig. 7]. The overall signal strength was greatest for the lower molecular weight markers and lowest for Penta D and Penta E.

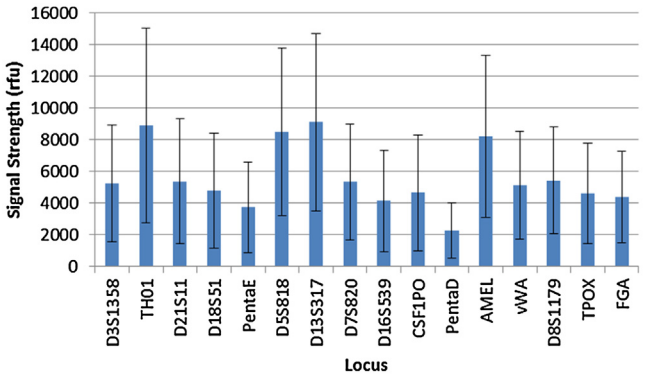

Fig. 7. Average signal strength in rfu at all loci with associated standard deviation for 1362 accuracy buccal swab samples.

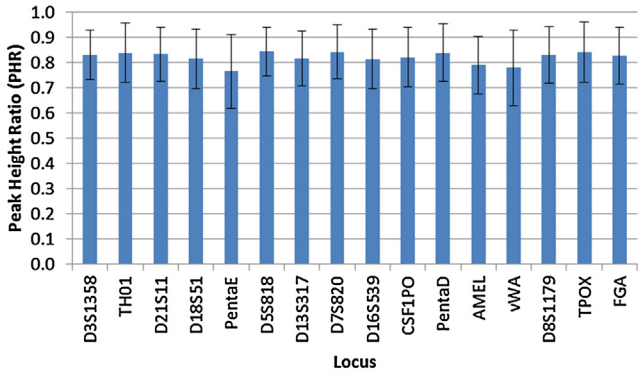

Fig. 8. Average peak height ratio by locus with standard deviation for 1362 accuracy buccal swab samples.

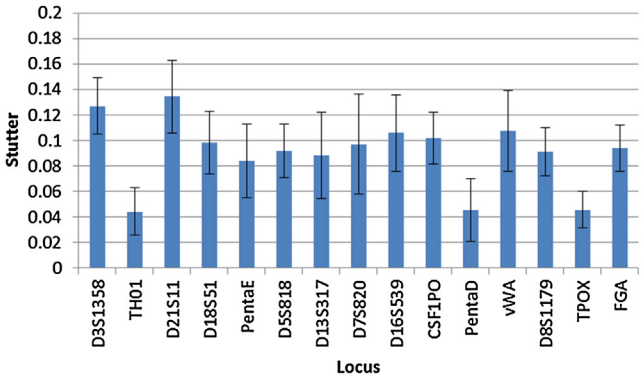

Fig. 9. Stutter ratio by locus with standard deviation for 1362 accuracy buccal swab samples.

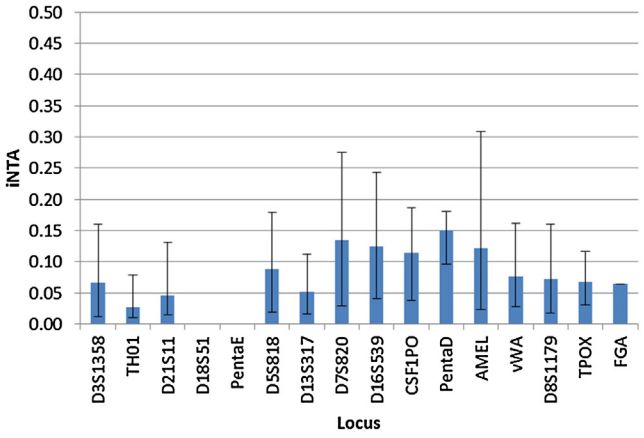

Fig. 10. iNTA ratio by locus with standard deviation for 1362 accuracy buccal swab samples.

3.7.4. Peak height ratio

The peak height ratio for each heterozygous locus was determined for each of the 1362 donor samples by dividing the signal strength of the weaker peak by the signal strength of the stronger peak. The peak height ratio ranges from 0.76 at Penta E to 0.84 at TH01, D5S818, D7S820, Penta D and TPOX [Fig. 8].

3.7.5. Stutter

The percentage of stutter at each locus was determined for 1362 accuracy samples by calculating the ratio of the peak height of the minor peak over the peak height of the called allele. The minor peak was identified as the peak 4 bases smaller than the called allele for tetranucleotide loci or 5 bases smaller than the called alleles for pentanucleotide loci. The average stutter varied from a low of 4.4% at TH01 to a high of 13.4% at D21S11 [Fig. 9].

3.7.6. Non-template addition

The percentage of incomplete non-template addition (iNTA) at each locus was determined for 1362 accuracy samples. Non-template addition was calculated as the ratio of the peak heights of the minor peak over the peak height of the called allele. The minor peak was identified as 1 base smaller than the called allele. The average varied from a low of 0% at D18S51 and Penta E (no iNTA observed) to a high of 15% at Penta D [Fig. 10].

3.7.7. Success rate

The first pass success rate, or the frequency that the first attempt to generate an STR profile was successful using the Expert System and/or manual review, was calculated for samples containing the CODIS core 13 loci. Eighty-four percent (84%) of the samples passed all expert system rules and yielded passing profiles with the CODIS core loci with no human review and an additional 7% of the profiles were deemed passing after human review, yielding an overall success rate of 91%. All profiles marked for human review by the Expert System were passed following the review.

The first pass success rate for the same set of samples processed by conventional STR typing was 87%.

3.7.8. Resolution

The evaluation of resolution looks at the system's ability to resolve a single base pair difference, such as the 9.3 and 10 alleles at TH01 [Fig. 11], as well as properly detect and label microvariants present within a locus.

Effective resolution was calculated based on the set of 1362 samples used for accuracy calculations [Fig. 12]. The equation for effective resolution was adapted by setting $R^* = 0.2$ as the measure of single base resolution [11].

$$R_{eff} = 0.2\frac{\Delta b(w1 + w2)}{2(t2 - t1)}$$

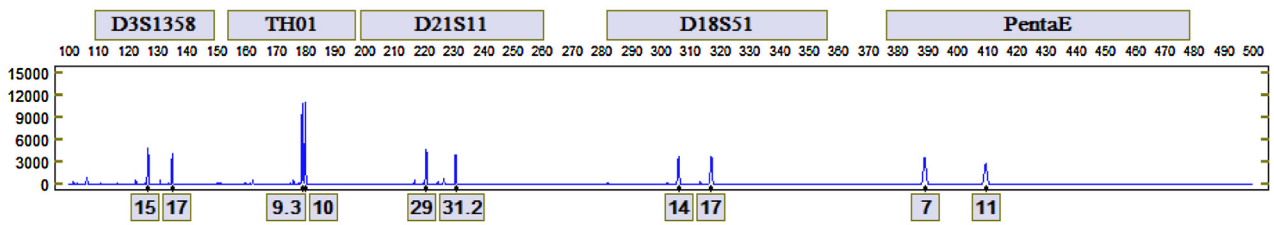

Fig. 11. Data for the Fluorescein channel of a sample which generated concordant allele designations of 9.3, 10 at TH01.

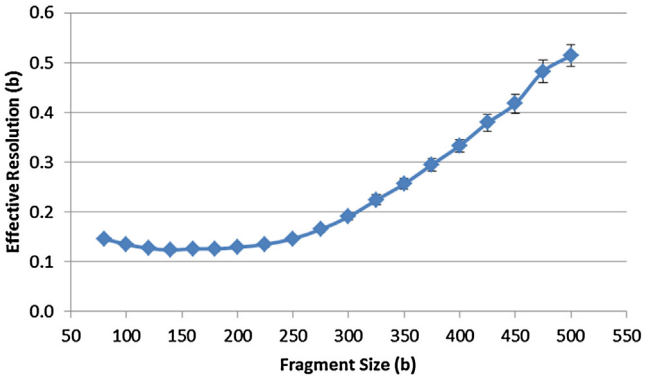

Fig. 12. Effective Resolution by fragment size in base pairs with standard deviation.

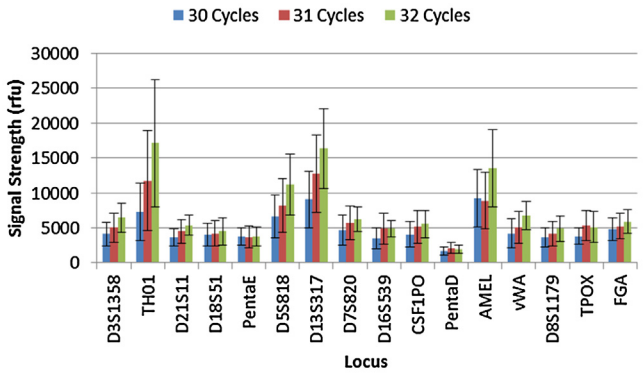

Fig. 13. Effect of 30, 31, and 32 cycles on signal strength and inter-locus signal strength balance.

In Gaussian Distribution :
$w = 4\sigma$, FWHM $= 2(\ln 2) \wedge 0.5 = 2.355\sigma$
$\sigma =$ Area$/[(2\pi) \wedge 0.5 *$ Height]

t – migration timew – baseline widthFWHM – full width at halfmaximumσ – standard deviationΔb – base pair difference

The results demonstrate the system has sufficient precision and resolution across all loci for effectively sizing microvariants present within all loci.

3.8. Contamination assessment

Eight (8) laboratories participated in this study and a total of 128 blank swabs were typed in four distinct patterns of alternating blank and buccal swabs [Table 1]. The blank swabs were new swabs removed from the packaging and placed directly into the cassette. The buccal swabs were swabs associated with another study, typically accuracy, and were collected in accordance with the procedure used for the corresponding study. All swabs in the alternating patterns were tested using the DNAscan System with integrated Expert System.

As expected, a small number of blank samples contained background noise or artifacts, however, none of the samples yielded any labeled alleles.

3.9. PCR-based studies

PCR studies of cycling parameters including anneal, denature, and extension times and temperatures, and cycle number were previously performed to optimize the reaction condition for a rapid microfluidic environment using a 7 μL reaction volume [7]. One study focused on the effect of cycle number on signal strength, inter-locus balance, and heterozygote peak height ratio. For this experiment, cycle numbers of 30, 31, and 32 were selected based on the thermal cycling guidelines provided in the Promega PowerPlex 16 System Technical Manual [13]. The overall signal strength increased by 23.5% and 23.9% when the cycle number was increased from 30 to 31, and 31–32 cycles respectively [Fig. 13].

Table 1
Description of the four configurations used in the study showing placement of blank swabs and buccal swabs.

	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
Pattern 1	Sample	Blank	Sample	Blank	Sample
Pattern 2	Blank	Sample	Blank	Sample	Blank
Pattern 3	Sample	Sample	Sample	Sample	Blank
Pattern 4	Blank	Sample	Sample	Sample	Sample

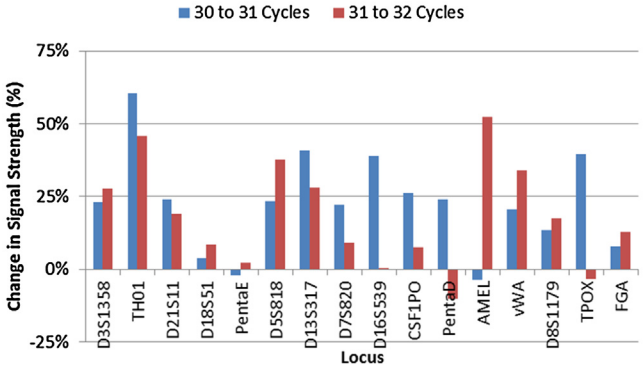

Fig. 14. Effect of signal strength when increasing cycle number from 30 to 31 and from 31 to 32.

The change in signal strength for each locus ranged from −2.1% and 2.3% for Penta E to 60.6% and 46.0% for TH01 [Fig. 14].

The data shows that inter-locus signal strength imbalance increases with increasing the cycle number and that peak height ratios remain relatively unchanged with cycle number [Fig. 15].

Thirty-one (31) cycles was selected because it provides the best combination of signal strength and inter locus signal strength balance.

4. The DNAscan expert system

The DNAscan Expert System Software processes the raw data, assigns allele designations, and employs rules to interpret the DNA

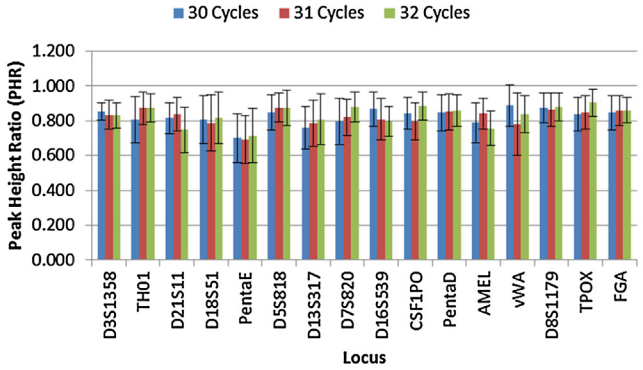

Fig. 15. Effect of 30, 31, and 32 cycles on peak height ratio.

profiles. The Expert System software was designed and developed for the analysis of DNAscan data and is fully integrated with no user intervention required. There are seven major Expert System steps required for STR profile generation on DNAscan [Fig. 16].

Immediately following a DNAscan run, optical data generated during electrophoresis is subjected to signal processing, which includes setting the baseline to zero and performing color correction. The Expert System then evaluates the internal lane standard and the allelic ladder using a strict set of criteria. Then, a series of rules are fired to assign alleles and evaluate locus and sample-specific criteria such as peak height, stutter, and heterozygote peak height ratio.

At the conclusion of the evaluation, the DNAscan Expert System generates the following outputs:

- Allele table listing all passing allele calls for all samples
- .png file (electropherogram) for rapid output visualization
- .xml file for upload to CODIS
- .fsa file to permit review with conventional software packages

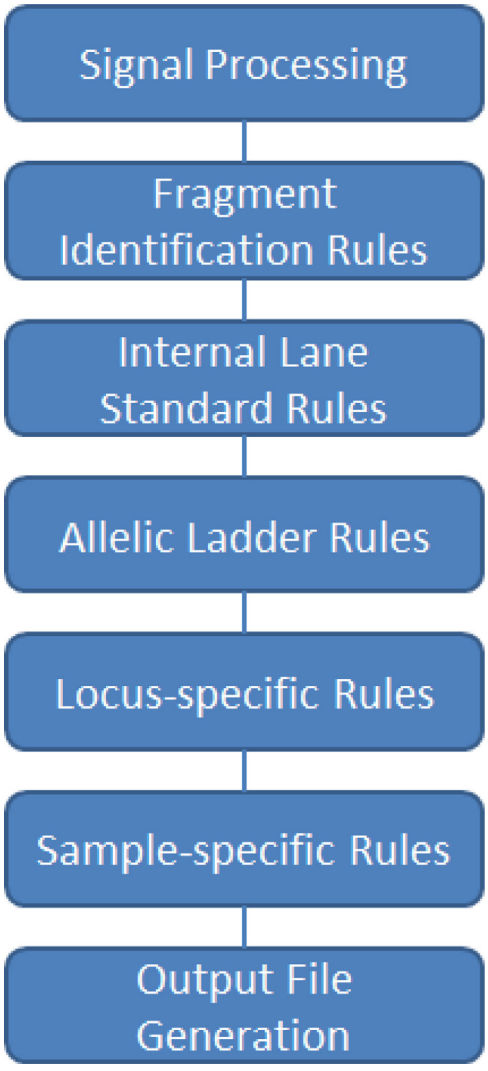

Fig. 16. Major steps performed by the DNAscan Expert System during STR profile generation.

5. Conclusion

Through an extensive developmental validation with external laboratories and over 2300 swabs tested, the DNAscan System has been shown to provide high quality, concordant results for reference buccal swabs, including automated data analysis with an integrated Expert System. Testing at eight sites demonstrates the reproducibility and reliability of the system and its successful use in different settings with numerous operators.

The DNAscan system shows minimal cross reactivity with other species, is resilient in the presence of numerous inhibitors, demonstrated an accuracy allele calling rate of 99.998%, and provided reproducible results for both buccal and purified DNA samples with sensitivity at a level appropriate for buccal swabs. The precision and resolution of the system is sufficient for detection of micro-variants and displays single base resolution. PCR-based studies provide confidence that the system is robust and that the amplification reaction has been optimized to provide high quality results. In addition to passing samples, the integrated Expert System appropriately identified and flagged samples with insufficient genetic information and failed mixed samples. The system meets the FBI's definition of Rapid DNA Analysis by performing "automated extraction, amplification, separation, detection and allele calling without human intervention" [1] and the system is easy-to-operate, utilizing a single room temperature-stable consumable per run.

In conclusion, the DNAscan Rapid DNA Analysis System, which provides "swab in – profile out" integrated STR profiling, has been shown to be robust, reliable, and is suitable for use in forensic human identification of single source reference sample buccal swabs.

The Developmental Validation of a fully integrated Rapid DNA Analysis System with Expert System data interpretation and subsequent formal National DNA Index System (NDIS) approval will allow forensic laboratories to utilize the technology for direct upload of reference sample buccal swab STR profiles to NDIS, a milestone in the responsible adoption of Rapid DNA Analysis. This critical first step will enable forensic laboratories to design effective and fundamentally sound Rapid DNA programs throughout their states. Ultimately, it is hoped that in tandem with appropriate legislative, policy, and infrastructure changes, this incremental implementation in the accredited forensic laboratory will lead to the use of Rapid DNA analysis in law enforcement booking stations. In addition to law enforcement applications, Rapid DNA analysis is being considered or applied in field forward military, disaster victim identification, and immigration settings. The completion of the DNAscan developmental validation adds support to both current and future applications of Rapid DNA Analysis to improve societal safety.

Competing interests

Eugene Tan, Rosemary Turingan, Catherine Hogan, and Richard Selden are employees of and shareholders in NetBio. Julie French is an employee of GE Healthcare Life Sciences. No non-financial conflicts of interest exist for any of the authors.

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