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DNA typing can be a labor intensive and time-consuming process which, even in ideal situations, can take up to approximately one full day from collected swab to generated genotype profile. To expedite the process from sample-to-result, automated rapid DNA typing platforms have been developed. These turnkey systems offer the potential of reducing the time from reference sample collection to fully-interpreted profile in less than two hours. However, for full consideration of rapid technology, results should be of equal quality to that of currently accepted methodologies. This project tested the hypothesis that automated rapid STR genotyping platforms perform as well as standard STR typing methods for typing reference samples by comparing the ability of NetBio's DNAscan™ automated rapid STR genotyping platform (NetBio, Inc., Waltham, MA) and standard STR typing methods to generate DNA profiles from reference samples. Multiple buccal swabs, collected from a number of individuals, were analyzed with the DNAscan instrument, an automated rapid STR genotyping platform that utilizes PowerPlex® 16 chemistry (Promega Corporation, Madison, WI). The rapid STR results were comparable with those obtained from the same individuals by standard STR genotyping processes also employing PowerPlex® 16 chemistry.

STR TYPING OF REFERENCE SAMPLES WITH RAPID DNA TECHNOLOGY

Andrea Moore, B.S.

APPROVED:

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Major Professor

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Committee Member

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Committee Member

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University Member

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Chair, Department of Molecular and Medical Genetics

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Dean, Graduate School of Biomedical Sciences

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STR TYPING OF REFERENCE SAMPLES WITH RAPID DNA TECHNOLOGY

THESIS

Presented to the Graduate Council

of the Graduate School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Andrea B. Moore, B.S.

Fort Worth, TX

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CHAPTER 1

INTRODUCTION

In the forensic DNA community, short tandem repeat (STR) analysis is widely used as a reliable tool for human identification. Present at about one in every ten kilobases (kb), STRs make up approximately 3% of the human genome and reside in both coding and non-coding regions (1-2). Because of the variation in number of repeats among individuals, forensically-relevant STRs are extremely useful for human identification. Additionally, because of their relatively small size, ranging from 100-400 base pairs, STRs can be amplified efficiently by a process commonly referred to as the polymerase chain reaction (PCR).

Traditional methods of DNA typing begin with the task of manually extracting the DNA from the sample and can be performed using several different approaches that are typically fall into one of two categories—solution-based or column-based (3). The steps involved in these extraction procedures generally consist of a cell lysis process, followed by inactivation of DNase enzymes, and separation of the nucleic acid from the remaining cellular debris (3).

One well-established, solution-based extraction method is often referred to as an organic extraction (3-4). This technique makes use of phenol-chloroform isoamyl alcohol (PCIA) to separate the DNA from proteins based on their differing solubility. Once the PCIA component is added to the solution, a hydrophobic layer which captures the proteins, lipids, carbohydrates, and cell debris settles to the bottom of the solution, while an hydrophilic

layer, which captures the nucleic acids, is simultaneously formed at the top of the solution. The DNA is collected from this aqueous layer and used for analysis (3-4).

While extraction using PCIA is a reliable, widely-accepted technique, it has some drawbacks. Of greatest concern is the fact that working with PCIA introduces some safety concerns. Phenol itself is a toxic carbolic acid that is flammable and highly corrosive. Chloroform causes respiratory tract irritation if inhaled and, if at large enough concentrations, is capable of causing central nervous system effects such as headaches, dizziness, unconsciousness, and coma. Special precautions must be taken when working with these chemicals (3, 5-6).

Partly due to these concerns, alternate extraction techniques have been developed, many of which fall into the category of solid-phase extraction procedures. Often utilizing silica matrices or anion-exchange carriers, solid-phase purification methods are provided in commercially available kits. These kits tend to utilize the same basic principles to adsorb nucleic acids from the extraction process in a chaotropic environment where hydrogen-bonding interactions occur that bind nucleic acids, wash away remaining denatured proteins along with other contaminants, and elute DNA (3,7).

Following DNA extraction, quantification is performed prior to the amplification step. Because genotyping assays function best within a specified range of template DNA input, it is important to measure the amount of DNA present in a sample (8). DNA quantification allows confirmation of both the presence and amount of DNA in a sample so that downstream analysis can be accomplished effectively and efficiently. Real-time quantitative PCR (qPCR) is the preferred method for DNA quantification (9). The process of qPCR uses nucleic probes, which have both a reporter dye and a quencher attached, that recognize specific markers. Once

hybridized to the target, a DNA polymerase digests the probe. The separation of the reporter dye from the quencher leads to an increase in fluorescent intensity, which is directly related to the amount of target DNA (10). (8-9). The entire qPCR process typically takes about 2 hours to complete.

The amplification process of targeting a specific region on DNA and exponentially increasing the number of particular fragments from that region is referred to as PCR (11). During this process, fluorescently labeled primers hybridize to flanking regions of specific STR loci, and through the use of a thermostable polymerase, these portions of the DNA are extended to generate multiple copies (11-13). With the use of a thermal cycler, PCR amplification follows three basic steps which are repeated through a series of cycles. The initial step involves exposing the double-stranded DNA to a high temperature (~95°C), causing the molecule to denature into two single-strand molecules. Once the DNA is denatured, the temperature in the PCR is reduced to approximately 50-70°C so that the primers can anneal to the template in the second step of a PCR cycle (13). In the third step of PCR, the temperature is raised to 72-80°C, facilitating the DNA polymerase (Taq) to add nucleotides to the extending primers based on complementary binding with the template target. In theory, the amount of target DNA generated during each cycle is essentially doubled (13).

In place of the extraction process described above, direct amplification can be used for typing samples such as reference buccal swabs. This method does not require a DNA purification step, allowing one to process samples much more quickly and efficiently (14). Direct amplification is performed when a sample is directly added to the PCR, and PCR amplification is carried out with only a modification on the initial denaturation step (15). Samples are heated for three minutes at 94°C and cooled for three minutes at 55°C. This two-step incubation period is

repeated three times in place of the usual one-step five minute incubation period at 94°C that is performed during traditional PCR amplification (15).

Once amplification is complete, capillary electrophoresis (CE) through the use of instruments, such as the 3500xL Genetic Analyzer (Life Technologies, Foster City, CA), is used to separate and detect the different target fragments that were generated during amplification (16-19). The fluorescent labels that are present on the primers incorporated into the amplicons during PCR enable detection of the STR amplified products (16-19). For CE, negatively-charged DNA fragments are injected electrokinetically into a capillary where they are separated by size through a liquid polymer sieving medium (16, 18-19). During their electrophoretic migration, the amplicons cross a path where a laser can excite the fluorescent dyes attached to each fragment, and the emission spectra generated from each dye are separated by a diffraction system, allowing for the detection and differentiation of each of the loci. Multiplexing makes use of 4-6 different fluors so that STRs of the same size are distinguished from one another, allowing for multiplexing of a larger number of STRs according to both size and specific fluor attached to the amplicon. Some STR loci are labeled with different fluorophores that emit light at differing wavelengths as a result of laser excitation; thus, detection and differentiation of all loci is possible (18). The resulting fluorescent signals are separated by a diffraction system and detected via a CCD camera (18), enabling each STR locus to be detected and distinguished from one another (Figure 1). The signals are converted into digital data, so that the results can be analyzed with software that is specifically designed for the task (17-18).

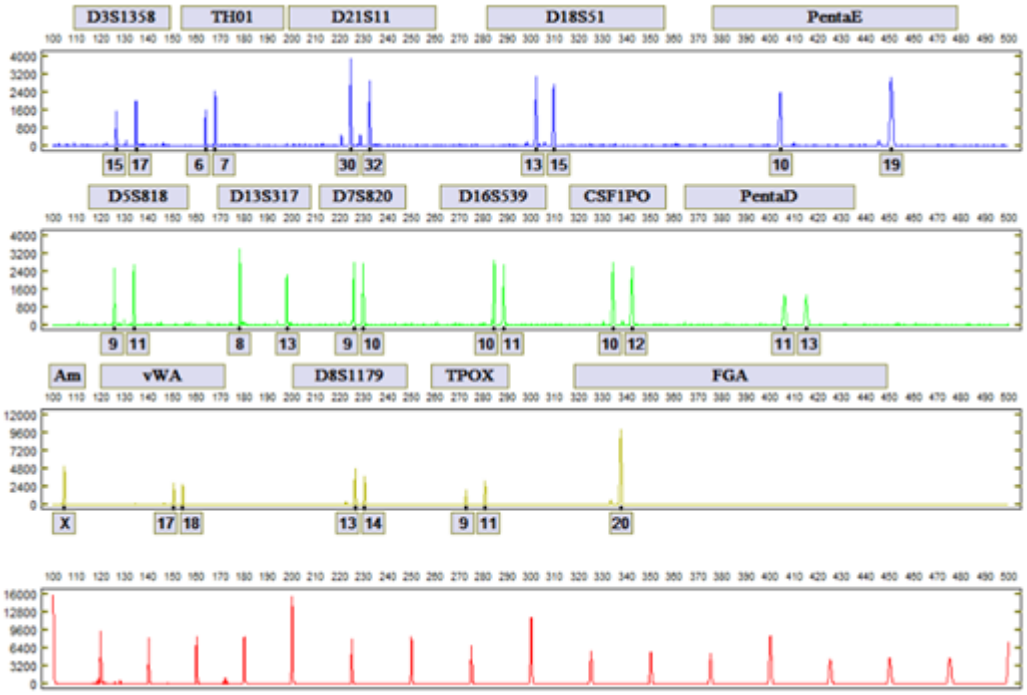


Figure 1: Electropherogram of a STR profile showing the various dye colors used to distinguish the loci. The respective loci and their alleles can be seen at each locus along with labels indicating the number of STR units corresponding to each allele.

Statement of Problem

In recent years, a number of advancements such as the incorporation of robotic platforms and direct amplification have played key roles in expediting this process. However, when using most current methods, one still may wait for a period of time that may take one to a few days before obtaining final interpreted results from STR genotyping processes. To overcome this lag time, decrease the amount of labor involved, and aid in expediting the overall analysis process, rapid STR genotyping platforms have been developed that enable a buccal swab to be analyzed in a completely automated fashion. These platforms integrate the multiple steps involved in STR genotyping, excluding the quantitation step as it is not required for reference samples because the

source is known and bacterial and fungal contamination may not be a significant constraint (19-23).

Research Significance

Tan et al (24) recently described generation of a fully automated platform, DNAscan (NetBio, Inc.), that is capable of processing buccal swab samples from start to finish, yielding a full STR profile in only 84 minutes (Figure 2). All processes are contained within a chip that includes purification, bead-based normalization, PCR, CE, and laser induced fluorescent detection. This system makes use of solid-phase nucleic acid extraction based on a purification process using guanidinium-based cell lysis and silica binding, as well as STR amplification using the STR primer sequences from the PowerPlex® 16 kit. Included in the instrument is a pneumatic subsystem that drives fluids throughout the BioChipSet cassette (BCSC), a thermal subsystem allowing for multiplexed STR amplification, a high-voltage subsystem for CE, and an optics subsystem used to excite and detect fluorescently-labeled STR fragments during electrophoresis (Figures 3 and 4). The instrument is ruggedized to MIL-STD 810F ensuring that it can be transported readily without the need for recalibration or optical realignment, making it well-suited for operation in a field-forward capacity. (24). Additionally, all reagents needed to perform STR genotyping are incorporated onto the BCSC in lyophilized form, allowing for the cassettes to be maintained at room temperature up to six months. The purpose of the project herein was to test the hypothesis that the DNAscan automated rapid STR genotyping platform performs as well as standard STR typing methods for typing reference samples. This will be done by comparing the ability of this automated rapid STR genotyping platform and standard STR typing methods to generate quality DNA profiles from reference samples.

Figure 2: Image of NetBio DNAscan fully integrated instrument obtained with permission from the GE Healthcare Life Sciences product catalog (25).

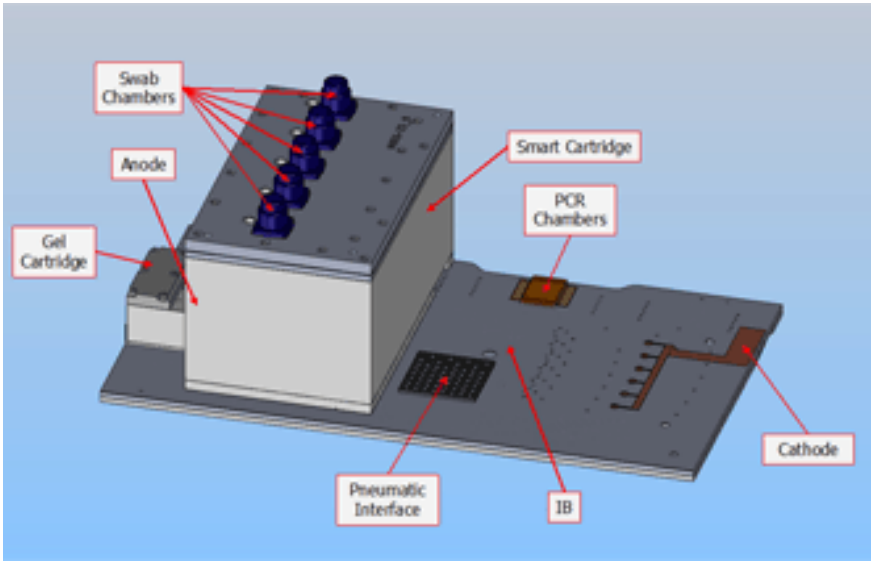


Figure 3: An image of a BSCS. The BSCS consists of five swab chambers and incorporates all the subsystems needed to complete DNA extraction through STR amplification and genotyping (26). Image was obtained with permission from GE Healthcare Life Sciences.

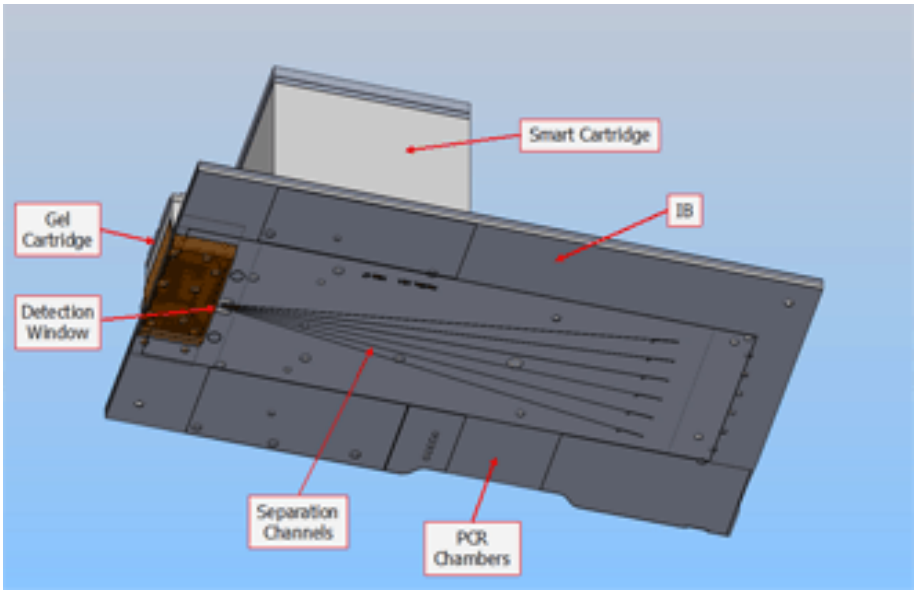


Figure 4: Image, obtained with permission from GE Healthcare Life Sciences, showing the underside of the BSCS which incorporates the separation channels, PCR chambers, and detection window (26).

CHAPTER 2

MATERIALS AND METHODS

Samples and Sample Preparation

NetBio BioChipSet Swabs (The Bode Technology Group, Lorton, VA) created specifically for the DNAscan instrument were provided by NetBio, Inc. (Waltham, MA) along with the BCSCs and the DNAscan instrument. A total of 65 buccal swabs were obtained from both male and female individuals. Puritan® Cotton Tipped Applicators (Puritan Medical, Guildford, ME) were used to collect an additional buccal swab from each of twenty individuals for concordance studies using traditional bench-top extraction and capillary electrophoresis methods. All samples were collected in accordance with approved UNTHSC IRB guidelines.

Sample Analysis

1. Sensitivity Study

   This experiment was designed to test the impact of different buccal swab collection techniques on the performance of the system when generating DNA profiles. For this study, "heavily collected" was defined as six swipes on a cheek with an up-and-down motion constituting one complete swipe, and "lightly collected" was defined as three swipes on a cheek with an up-and-down motion constituting one

complete swipe. Buccal swabs were collected from a total of twenty different individuals who were each swabbed twice. One of the two swabs was used to obtain a "heavily collected" sample while the other swab was used to obtain a "lightly collected" sample, each of which were processed on the DNAscan instrument.

2. Reproducibility Study

This study was designed to test the precision of the system when generating DNA profiles. A total of twenty buccal swabs were collected from two different individuals. Each individual was swabbed ten different times, and the profiles were generated using the DNAscan instrument. The profiles were compared to determine the level of agreement among them.

3. Contamination Study

This experiment was designed to test whether any intra- or inter-run contamination occurs with the use of rapid DNA instrumentation. Ten buccal swabs were obtained from one individual to serve as known positive controls. During the first run, the samples were inserted into the BCSC in the following manner:

Blank	Sample	Blank	Sample	Blank

For the second run, the samples were inserted into the BCSC in the following manner:

Sample	Blank	Sample	Blank	Sample

The profiles were analyzed to determine whether any spurious alleles were present in the negative control swab chambers or in the positive controls, which would indicate that contamination occurred.

4. Inhibition Study

To study the effects of known inhibitors on buccal swab reference samples run on the DNAscan instrument, buccal swabs were collected from five different individuals who consumed hamburgers and soft drinks just prior to swabbing. These profiles were evaluated to determine whether there was indication of inhibition in the form of allele drop-out.

Concordance Study

1. Sample Collection

Using Puritan® Cotton Tipped Applicators, buccal swabs were obtained from twenty different individuals whose STR profiles were generated during sensitivity studies on the DNAscan instrument. The samples were allowed to air-dry and were maintained for two months in separate paper wrappers prior to the extraction process.

2. DNA Extraction

Samples analyzed by the standard method were extracted using a solid-phase extraction method with the QIAGEN® QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's recommended protocol. Each swab was placed in a 2 mL microcentrifuge tube, and 400 µL of PBS were added to each. Twenty µL proteinase K and 400 µL Buffer AL were added to each sample, and the samples were mixed immediately via vortexing for 15 s. Each sample was incubated at 56°C for 10 min followed by a brief centrifugation step at 2000 x g for 10 s to remove any drops from the lids. To each sample, 400 µL of 100 % ethanol were

added. The samples then were mixed by vortexing and centrifuged at 2000 x g for 10 s to remove any drops from the lids. Following this step, 700 µL of this mixture were added to the QIAamp Mini spin column in a 2 mL collection tube. Each sample was placed in a centrifuge at 6000 x g for 1 min. The QIAamp Mini spin columns were each placed into clean 2 mL collection tubes, and the tubes containing the filtrate were discarded. This step was repeated by applying 700 µL of the remaining mixture from above ethanol step to the QIAamp Mini spin columns. The QIAamp Mini spin columns were opened, 500 µL of Buffer AW1 were added to each, and the samples then were centrifuged at 6000 x g for 1 min. Each QIAamp Mini spin column was placed in a clean 2 mL collection tube while the collection tubes containing the filtrate were discarded. The QIAamp Mini spin columns were opened, 500 µL of Buffer AW2 were added to each column, and the samples were centrifuged at full speed for 3 min. To remove any AW2 carryover, each QIAamp Mini spin column was placed into a new 2 mL collection tube, the old collection tubes containing the filtrate were each discarded, and the samples were centrifuged at full speed for 1 min. Each QIAamp Mini spin column was placed in a clean 1.5 mL microcentrifuge tube, and the collection tubes containing the filtrate were discarded. The QIAamp Mini spin columns were opened, 150 µL of Buffer AE were added to each, and the samples then were incubated at room temperature for 1 min. The samples were centrifuged at6000 x g for 1 min each and stored at 4°C overnight.

3. DNA Quantification

The quantity of DNA present in each sample was determined using Quantifiler® Human DNA Quantification Kit (Life Technologies) and Applied Biosystems® Real-

Time PCR System (Life Technologies) using a reduced reaction volume protocol of 10 µL per sample. Standards were prepared using TE^{-4} buffer without glycogen. Nine tubes were labeled as follows: STD 1, STD 2, STD 3, STD 4, STD 5, STD 6, STD 7, STD 8, and NTC (No Template Control). To prepare STD 1, 10 µL Quantifiler® Human DNA Standard (200 ng/ µL) stock were added to 30 µL TE^{-4} buffer to obtain a total volume of 40 µL. A volume of 20 µL TE^{-4} buffer was added to each of the remaining STD 2-8 tubes as well as to the NTC tube. To the tube labeled STD 2, 10 µL of STD 1 were added to the 20 µL of TE^{-4} buffer, and using a serial dilution, the remaining standards were diluted by 10 µL in successive order. No DNA was added to the NTC tube. A master mix was prepared by adding the Quantifiler® Human Primer Mix and the Quantifiler® PCR Reaction Mix together in a microcentrifuge tube and vortexing them thoroughly. To each reaction well, 9.0 µL of PCR master mix were added along with 1.0 µL of the NTC, 9947A positive control, standard, or sample into the appropriate wells, yielding a final volume of 10 µL per well. Each standard was run in duplicate, as was the 9947A positive control at concentrations of 0.1 ng/ µL and 1.0 ng/ µL. The 96-well reaction plate then was covered with an optical adhesive cover and centrifuged at 2000 x g for about 30 s. The samples were placed on a 7500 Real-Time PCR System (Life Technologies) where the run was performed. The amplification parameters were as follows: 1 cycle of initial heating at 95°C for 10 min; 40 cycles of denaturation at 95°C for 15 s and extension at 60°C for 1 min.

4. STR Amplification

The DNA from each sample was normalized to 0.5 ng/µL so that 0.5 ng of DNA was added to the amplification reaction. The DNA from each sample was amplified using the PowerPlex® 16 HS Amplification Kit (Promega Corp., Madison, WI) on a GeneAmp® PCR System 9700 according to the manufacturers' recommended protocol. A PCR amplification mix was prepared by adding 5.0 µL per sample of PowerPlex® HS 5X Master Mix, 2.5 µL per sample of PowerPlex® 16 HS 10X Primer Pair Mix, and 16.5 µL per sample of amplification grade water to a sterile tube and vortexing the mixture for 10 s. The PCR amplification mix was dispensed by pipetting 24 µL of the amplification mix into the reaction wells of a 96-well reaction plate. To the appropriate wells on the amplification plate, 1 µL (0.5 ng) of each sample template DNA was added. Positive and negative amplification controls were added to the appropriate reaction wells on the amplification plate by adding 1 µL (0.5ng) of 2800M Control DNA and 1 µL of amplification grade water, respectively. Prior to placement on the GeneAmp® PCR System 9700, the plate was sealed and centrifuged for about 10 s to remove any air bubbles and bring the contents of each well to the bottom of the plate. Thermal cycling was completed as follows: heating the samples at 96°C for 2 min; then 10 cycles of ramp 100 percent to 94°C for 30s, ramp 29 percent to 60°C for 30s, and ramp 23 percent to 70°C for 45 s; then 22 cycles of ramp 100 percent to 90°C for 30 s, ramp 29 percent to 60°C for 30 s, and ramp 23 percent to 70°C for 45 s; then 60°C for 30 min followed by a 4°C soak for infinity until the samples were ready for further processing.

5. STR Data Analysis

STR genotyping was completed on a 3500xL Genetic Analyzer CE system (Life Technologies) in accordance with the manufacturer's recommendations. A total of 24 samples were injected in two separate injections for 24 s at 1.2 kilovolts with a 1500 second runtime. Fragment separation was carried out through the use of POP-4™ polymer (Life Technologies) and the HID36_POP4xl module (Life Technologies). Following sample processing, data analysis was performed using GeneMapper® ID-X Software v1.2 (Life Technologies). The detection threshold was set at 60 relative fluorescent units (RFU), and spectral artifacts such as stutter, shouldering, and pull-up were removed from each profile.

.CHAPTER 3

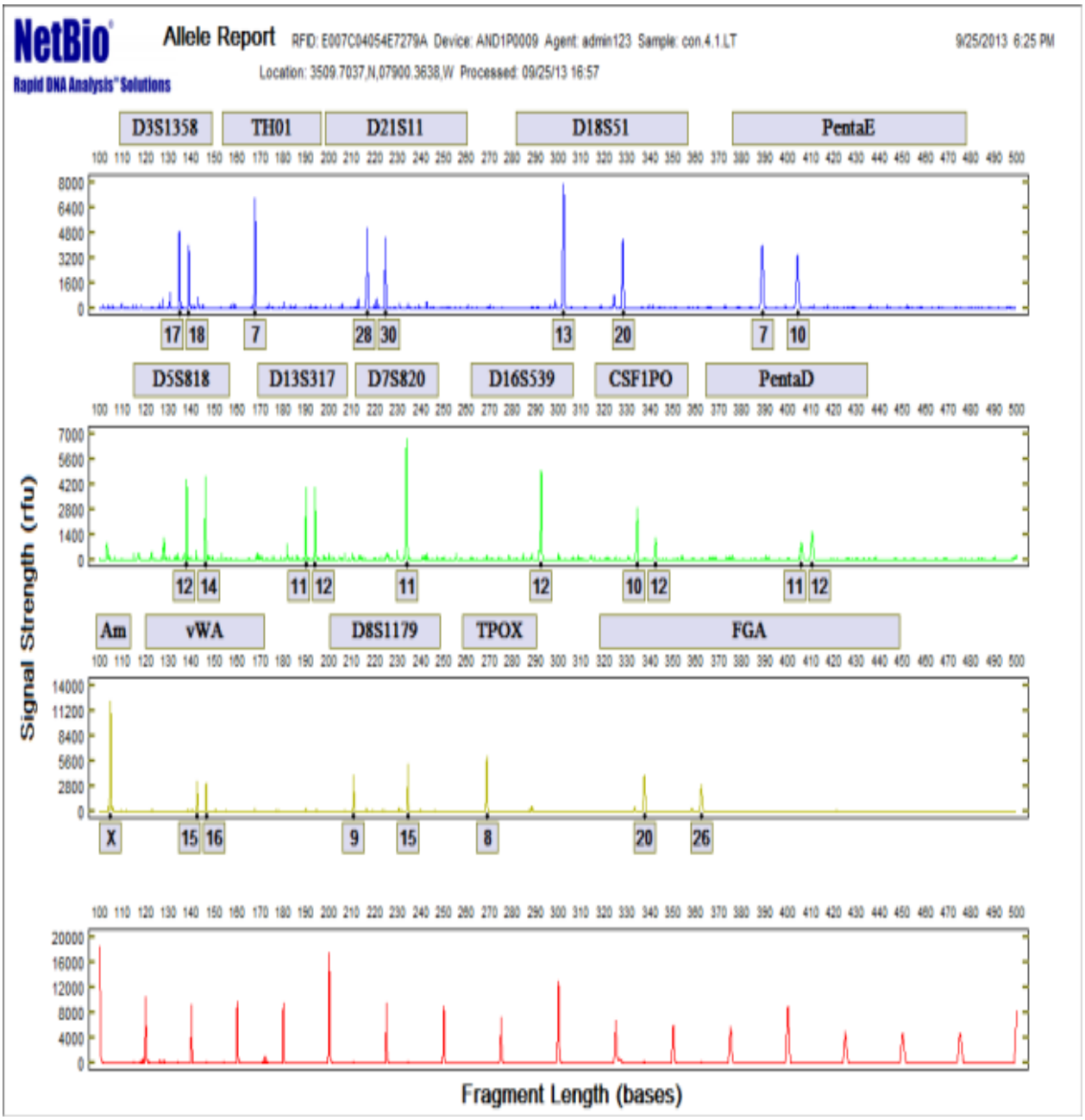
RESULTS

Sensitivity

A total of 40 buccal swabs was obtained from twenty individuals, each of whom was swabbed twice. One swab was a heavily collected sample, and the second swab was a lightly collected sample. The resulting profiles were evaluated based on peak heights, peak morphology, the presence of artifact, and evidence of allele drop-out. Each of the profiles generated from the lightly collected sensitivity samples were compared to the profiles generated from the heavily collected samples (Figure 5). Overall, the data indicated that full profiles could be generated with 85 percent of heavily collected buccal swabs and 95 percent of lightly collected buccal swabs on the DNAscan. The peak heights of all but one of the lightly collected samples were all well above the detection threshold of 150 RFU, and although one of the lightly collected samples showed some evidence of peak height imbalance at the FGA locus, defined as a peak height ratio less than 0.37, the remainder of these samples did not show any evidence of imbalance (Figure 6a-b). Two of the heavily collected samples generated profiles with peaks that fell below the detection threshold, and one of the heavily collected samples failed to generate a profile with any alleles (Figure 6c); but none of the heavily collected sampled exhibited any peak height imbalance. However, in all of these samples, the loci in question were flagged or called inconclusive by the DNAscan software, reducing the risk of mistyping the profiles.

Peak morphology was examined at all loci from the lightly collected and heavily collected samples. All allele peaks presented with good morphology, where alleles were called. Additionally, each of these samples was evaluated to determine the presence of artifact and baseline noise. Stutter could be seen in multiple profiles generated from both sample types, and shouldering was observed in a few of the samples from both the lightly collected and heavily collected sample types (Figure 7). However, these events are not uncommon when typing reference samples, regardless of the methods used, and the final profiles generated on the DNAscan were not compromised as a result of the stutter and shouldering that was noted. Although baseline noise was notable in several of the STR profiles that were generated from both lightly and heavily collected sample types (Figure 8), the final STR typing results were not affected by this occurrence.

To determine whether allele drop-out had occurred in any of the sensitivity samples, the STR profiles were examined for the occurrence of decreased peak heights across all loci. Some decreasing peak heights were observed in multiple samples, mostly at the Penta D and Penta E loci (Figure 9). In two of the heavily collected samples, drop-out was evident at various loci which were either missing one or both alleles (Figure 10); however, the instrument software flagged these samples as problematic, eliminating any possibility of mistyping, as these loci were not called. Additionally, low peak heights were evident at multiple loci in these samples, also indicating the possibility of drop-out issues; but again, these samples were flagged as problematic, preventing any mistyping. The results of the lightly collected sensitivity samples showed that one of these samples demonstrated drop-out at three different loci (Figure 11); however, all of these loci were flagged by the instrument.


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
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D7S820
D16S539
CSF1PO
PentaD
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11 12
11
12
10 12
11 12
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Signal Strength (rfu)
Fragment Length (bases)

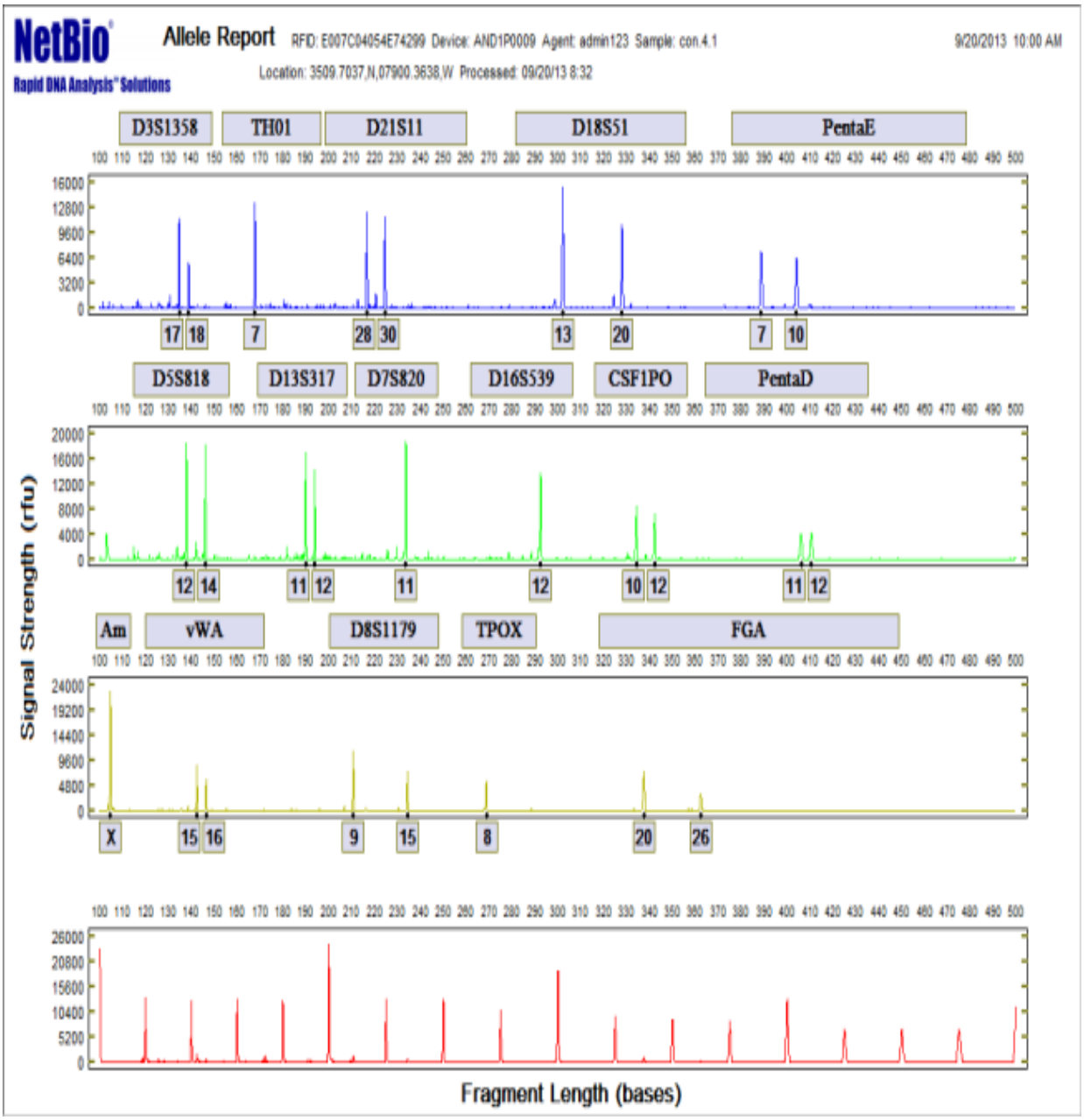
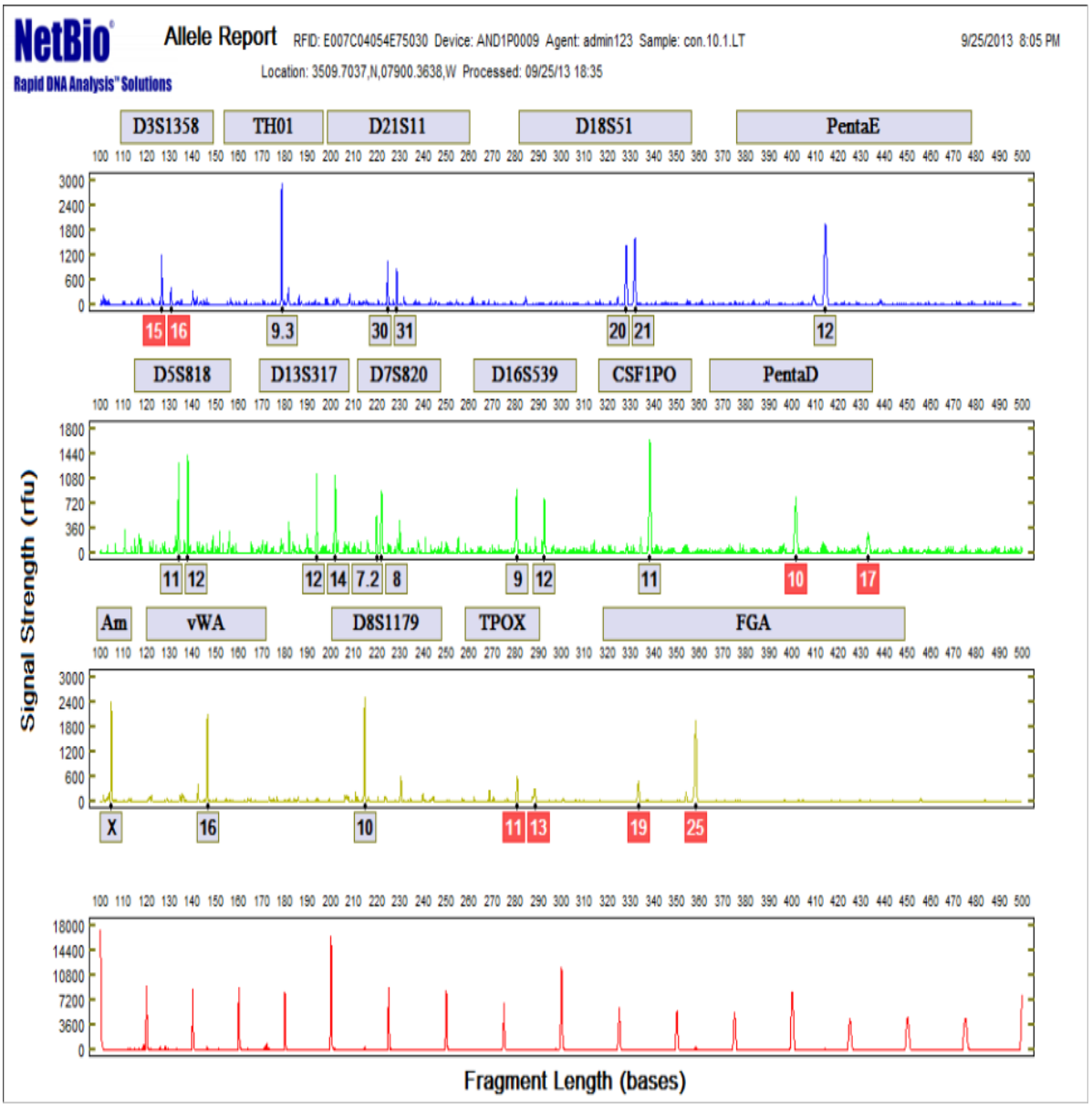


Figure 5: An example of an electropherogram from a lightly collected sample (top) compared to an electropherogram from a heavily collected sample (bottom) from the same individual. As was characteristic of profiles generated during the sensitivity study, the profiles seen here are similar.

(a)


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
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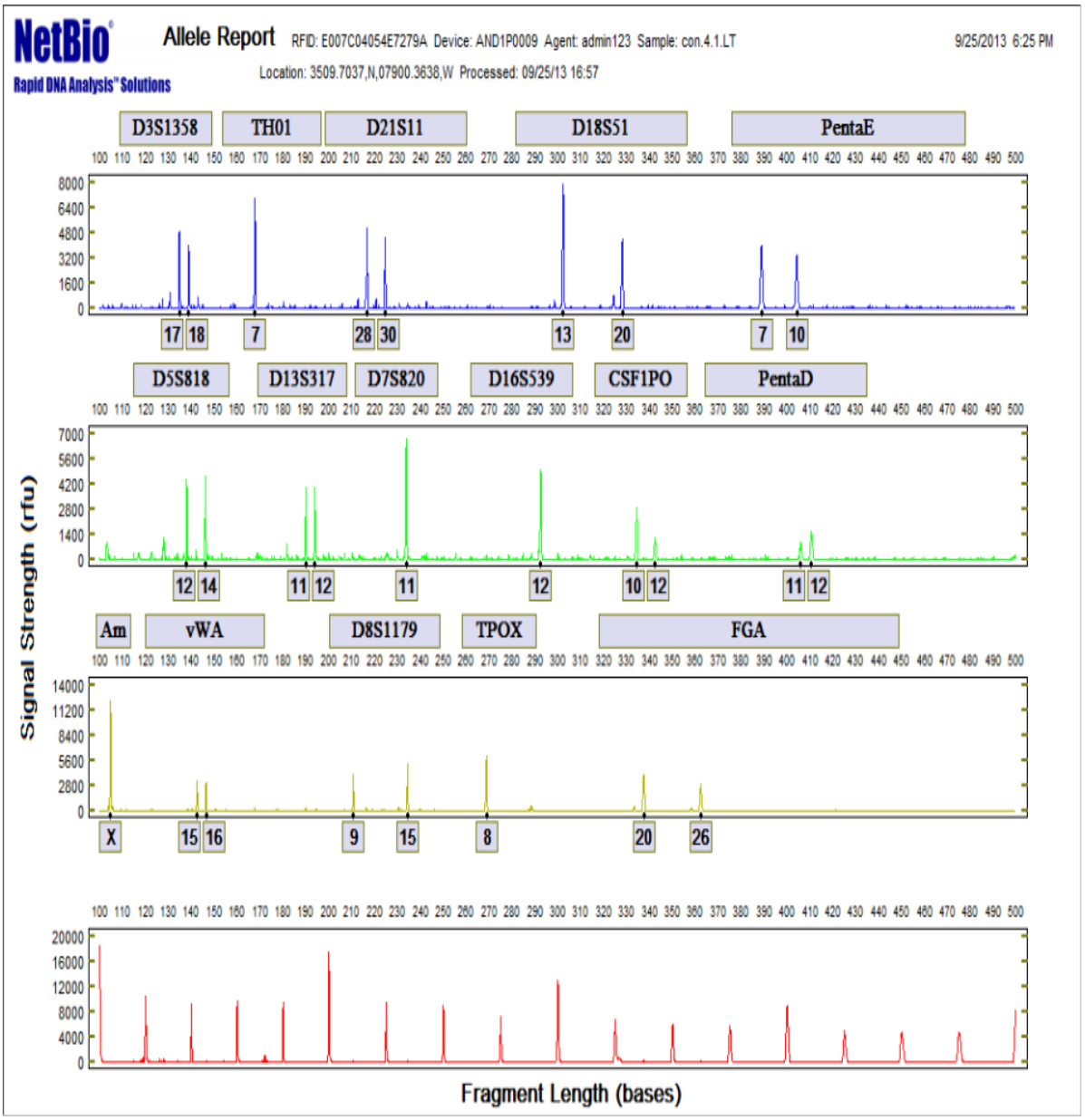
Location: 3509.7037,N,07900.3638,W Processed: 09/25/13 18:35
9/25/2013 8:05 PM
D3S1358
TH01
D21S11
D18S51
PentaE
15 16
9.3
30 31
20 21
12
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
11 12
12 14 7.2 8
9 12
11
10
17
Am
vWA
D8S1179
TPOX
FGA
X
16
10
11 13
19
25
Signal Strength (rfu)
Fragment Length (bases)

(b)


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
RFID: E007C04054E7279A Device: AND1P0009 Agent: admin123 Sample: con.4.1.LT
Location: 3509.7037,N,07900.3638,W Processed: 09/25/13 16:57
9/25/2013 6:25 PM
D3S1358
TH01
D21S11
D18S51
PentaE
17 18
7
28 30
13
20
7
10
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
12 14
11 12
11
12
10 12
11 12
Am
vWA
D8S1179
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FGA
X
15 16
9
15
8
20
26
Signal Strength (rfu)
Fragment Length (bases)

(c)

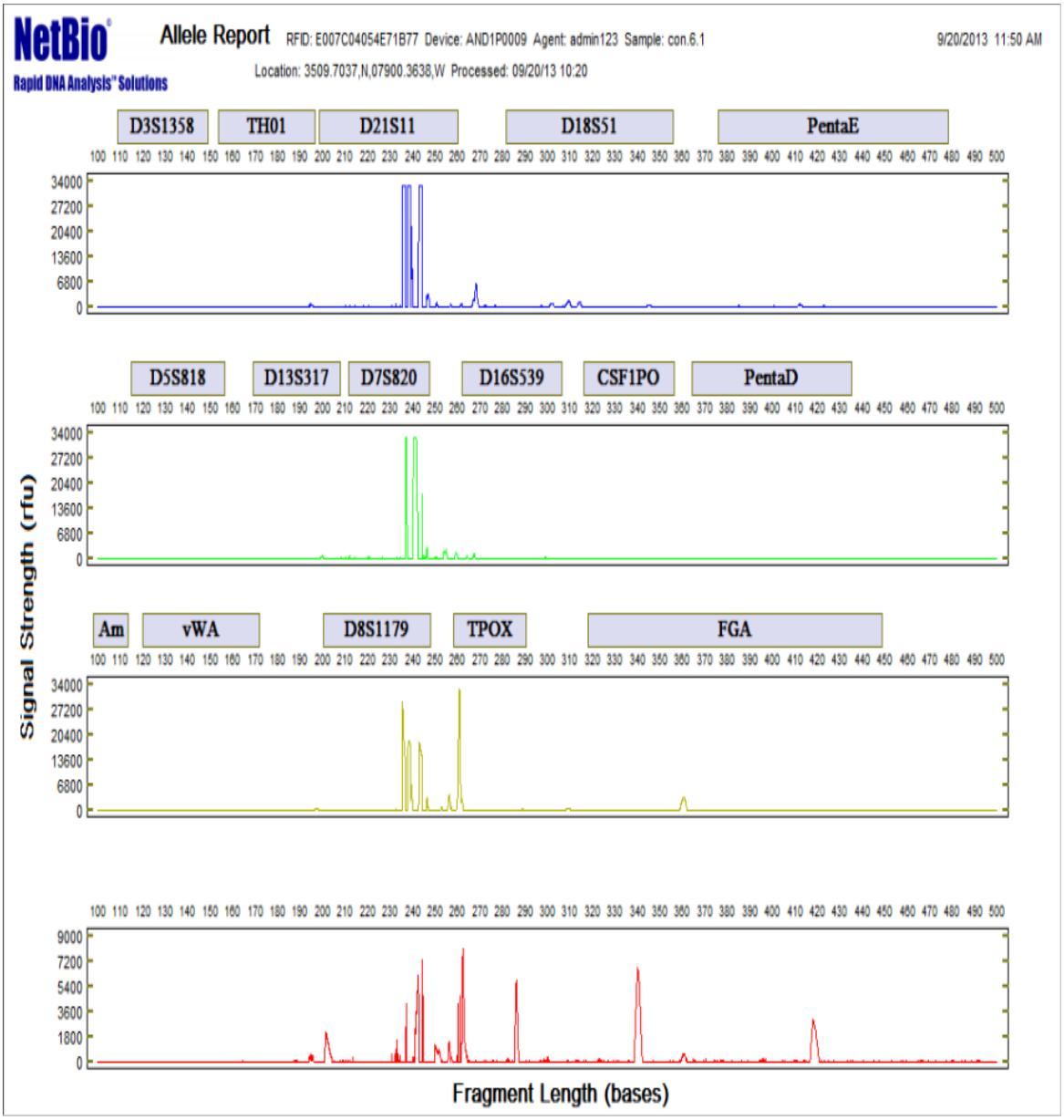


Figure 6: Three electropherograms obtained from two lightly collected samples (a-b) and one heavily collected sample (c). In the first profile, generated from a lightly collected swab, peak height imbalance can be seen at loci with alleles labeled in red (a). In the second profile, generated from a lightly collected swab, there is no evidence of peak height imbalance (b). One profile generated from a heavily collected sample failed to generate a profile (c).

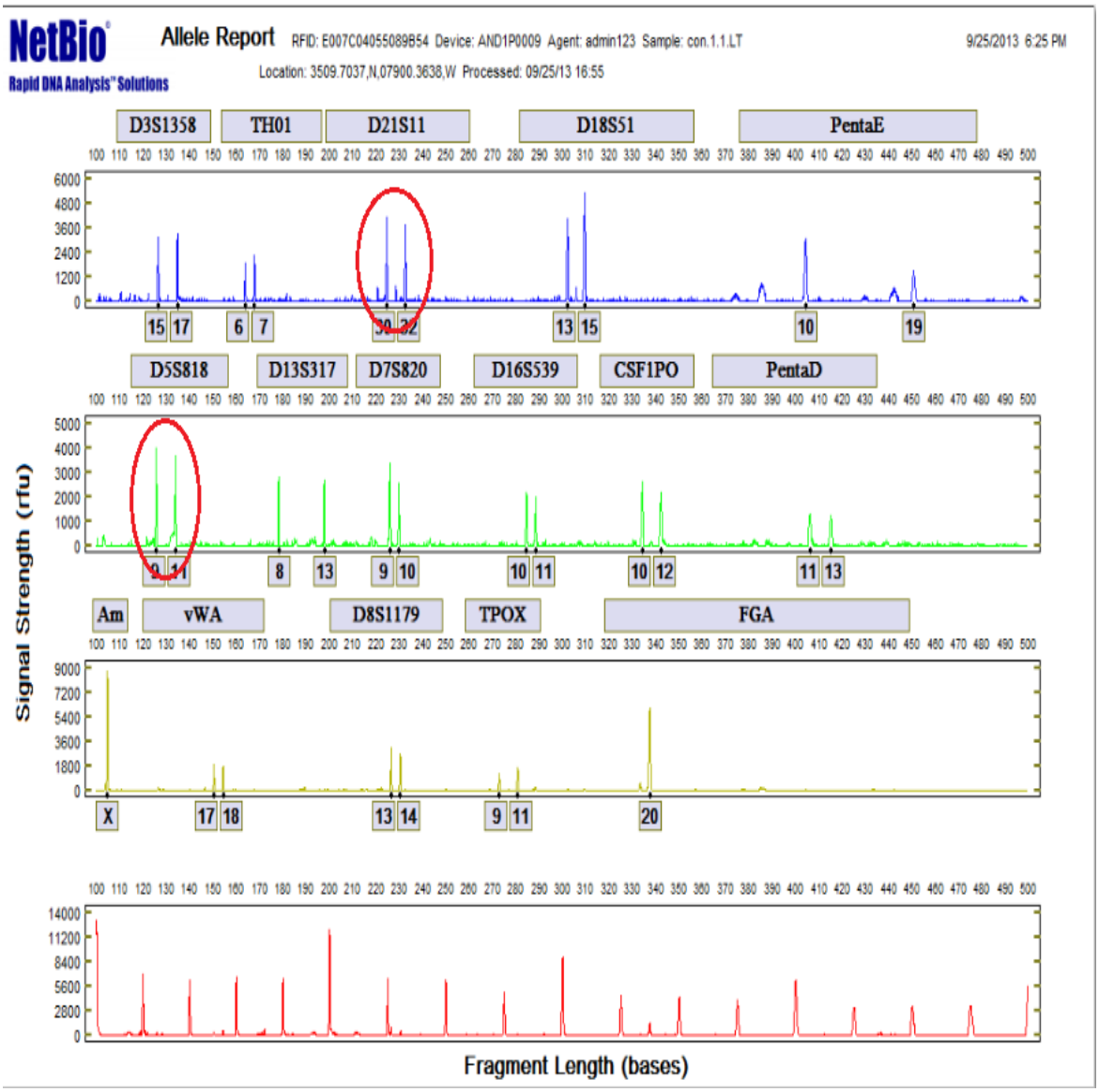
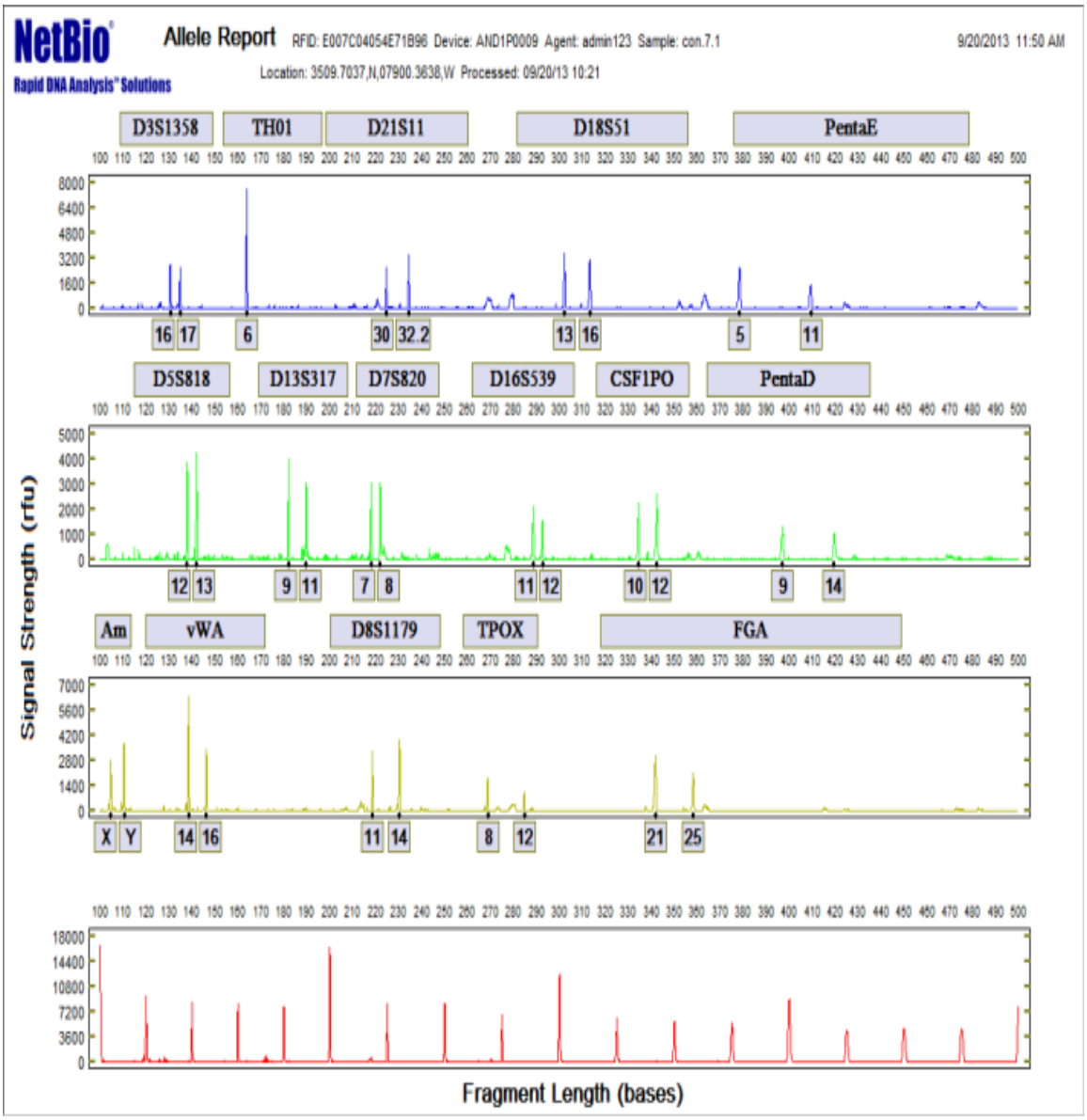


Figure 7: An electorpherogram generated from a lightly collected sample showing stutter peaks at D21S11 and shouldering at D5S818; however, these events did not alter the profile that was generated and do not pose a problem for DNA typing of reference samples.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TH01
D21S11
D18S51
PentaE
16 17
6
30 32.2
13 16
5
11
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
12 13
9 11
7 8
11 12
10 12
9
14
Am
vWA
D8S1179
TPOX
FGA
X Y
14 16
11 14
8 12
21 25
Signal Strength (rfu)
Fragment Length (bases)

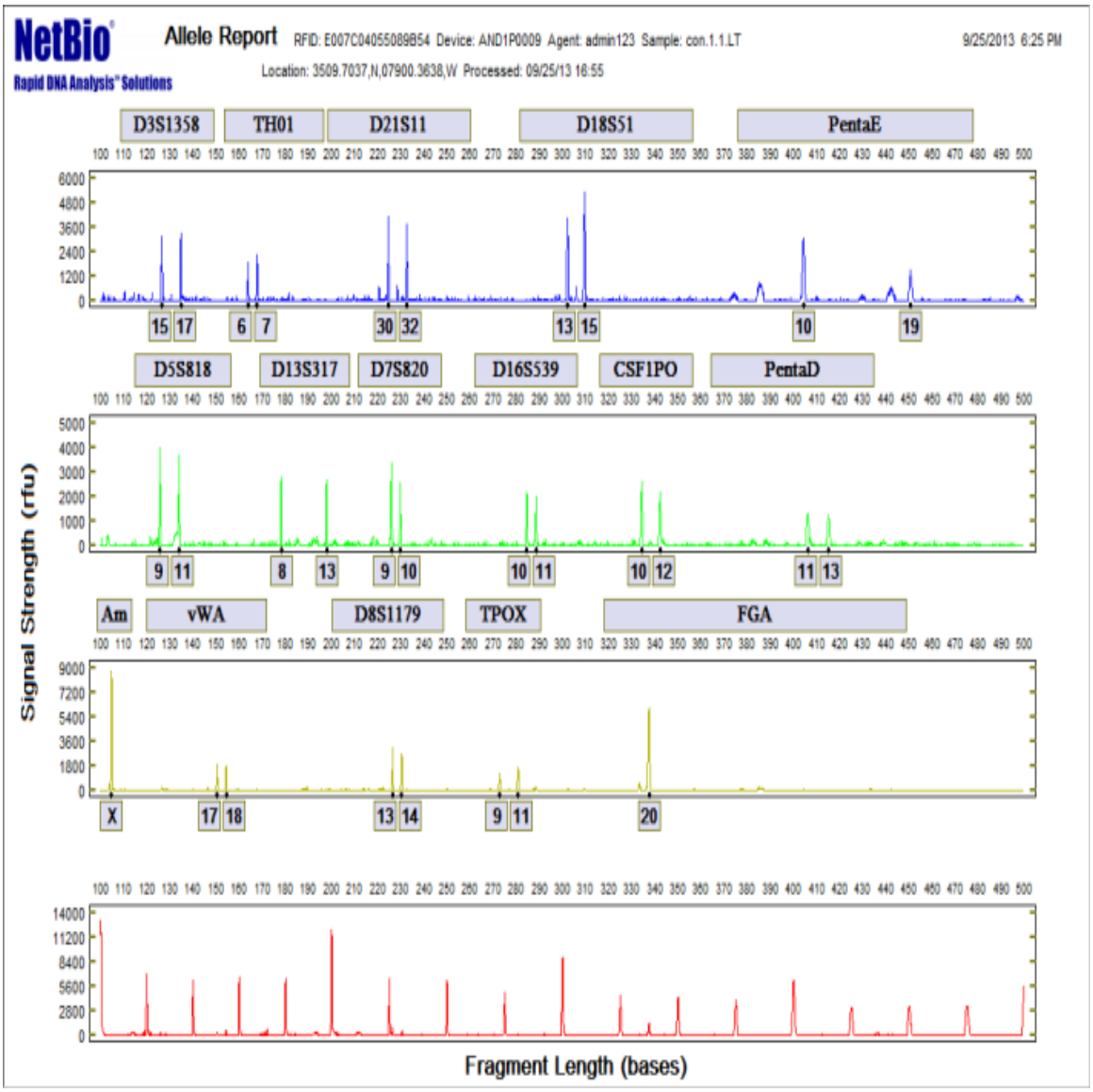
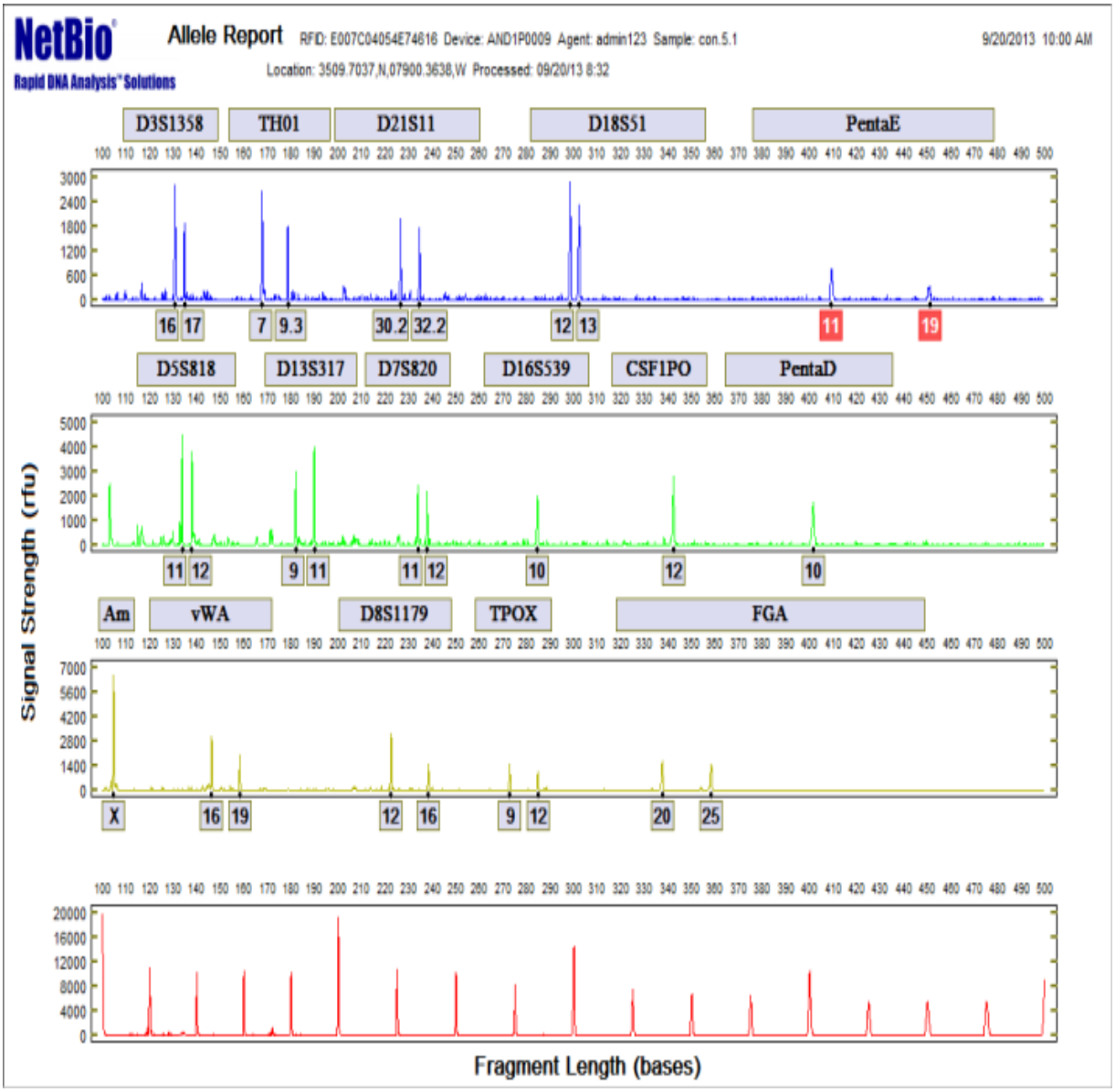


Figure 8: Two electropherograms, from a heavily collected sample (top) and a lightly collected sample obtained from a different individual (bottom), showing baseline noise.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Rapid DNA Analysis® Solutions
Allele Report
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9/20/2013 10:00 AM
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TH01
D21S11
D18S51
PentaE
16 17
7 9.3
30.2 32.2
12 13
11
19
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
11 12
9 11
11 12
10
12
10
Am
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TPOX
FGA
X
16 19
12
16
9 12
20
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Signal Strength (rfu)
Fragment Length (bases)

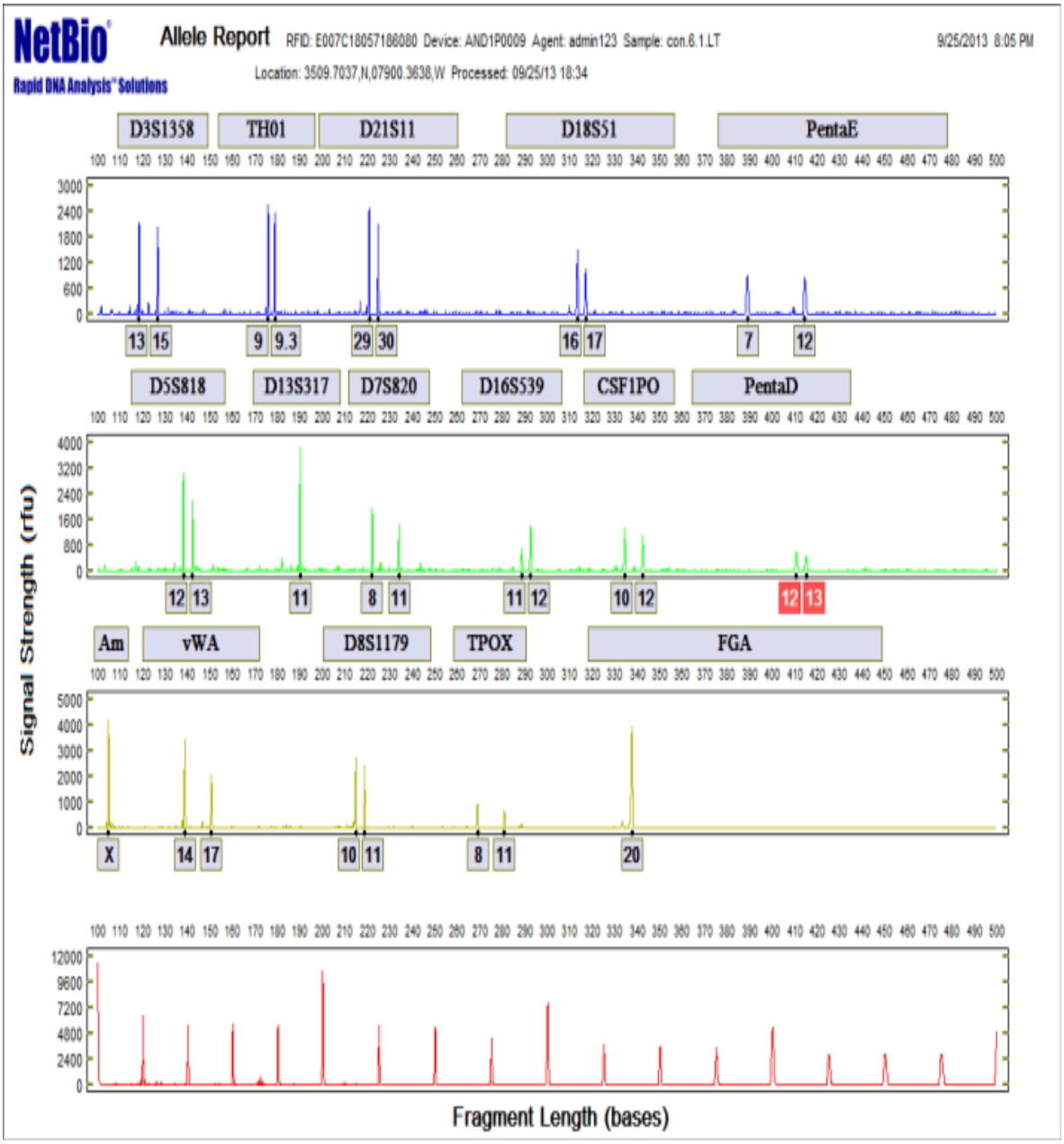
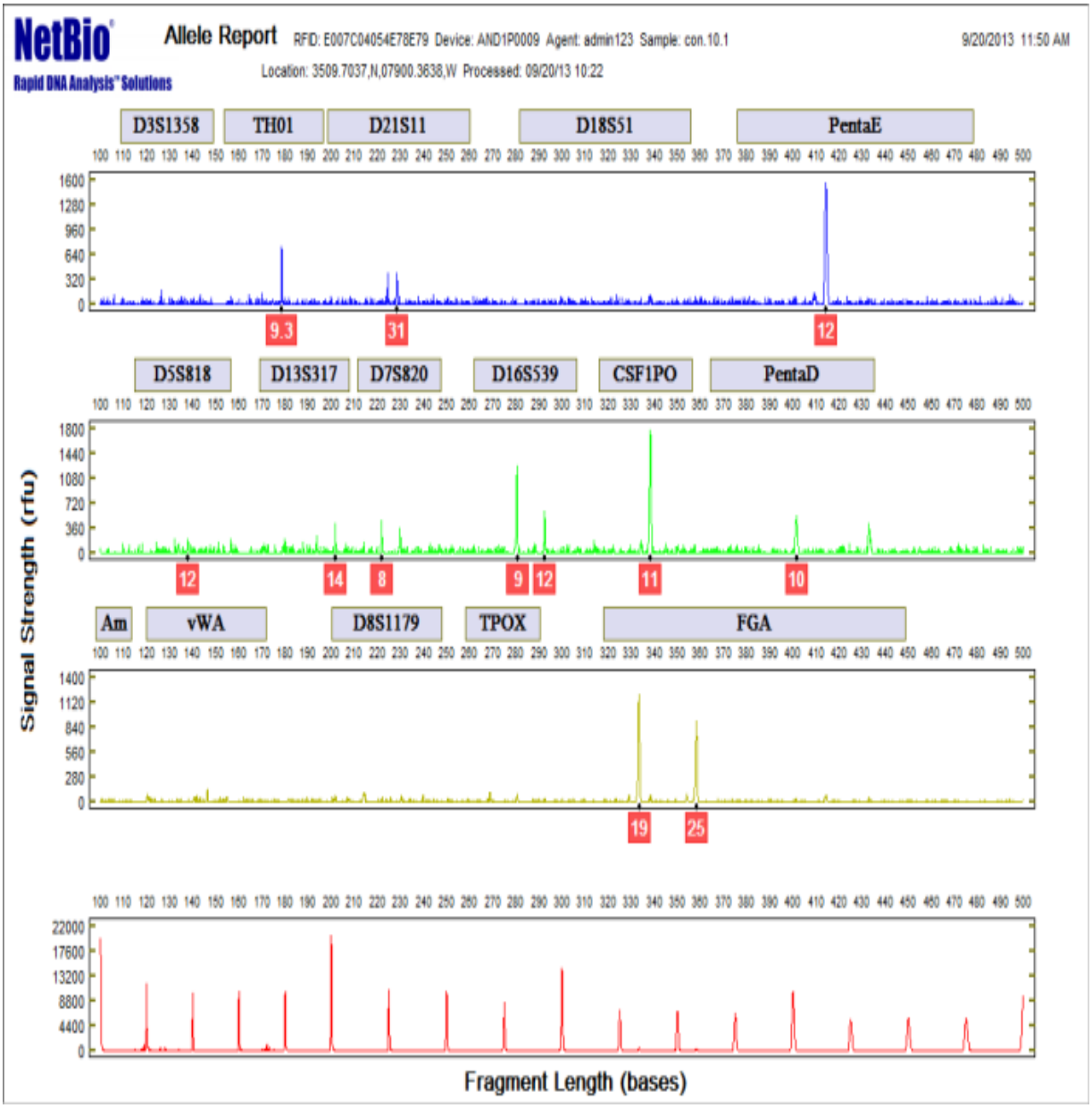


Figure 9: Two electropherograms, from a heavily collected sample (top) and from a lightly collected sample (bottom), showing decreased peak heights at Penta E and Penta D respectively.


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
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9/20/2013 11:50 AM
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TH01
D21S11
D18S51
PentaE
9.3
31
12
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
12
14
8
9
12
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TPOX
FGA
19
25
Signal Strength (rfu)
Fragment Length (bases)

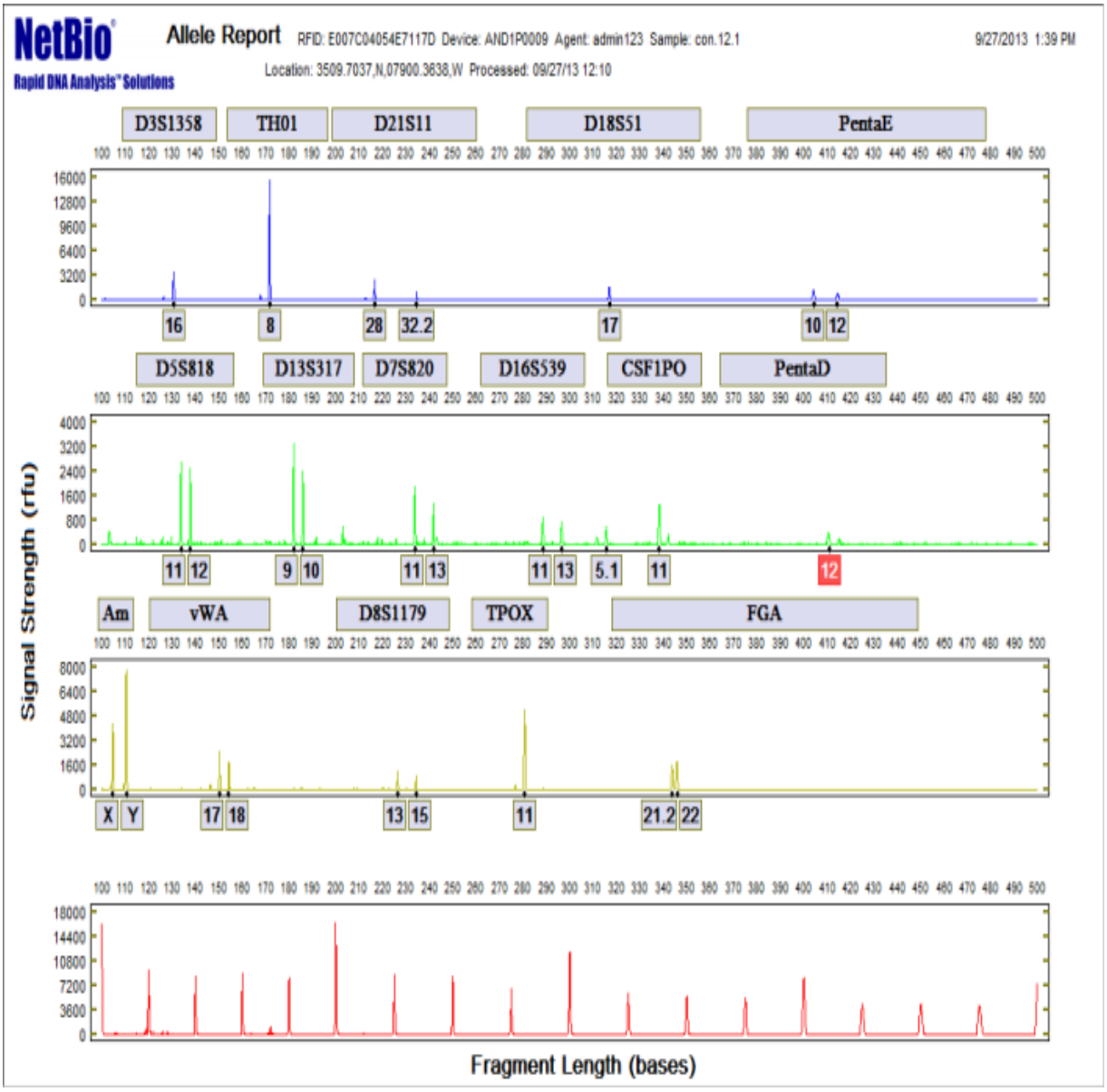


Figure 10: Two electropherograms generated from two heavily collected samples. The loci labeled in red indicate low peak heights and possible drop-out issues and, thus, would be deemed inconclusive. Some loci in the first profile (top) do not have any alleles present. Additionally, in the second profile (bottom), the second allele at the Penta D locus has dropped out, although this locus also would have been called inconclusive.

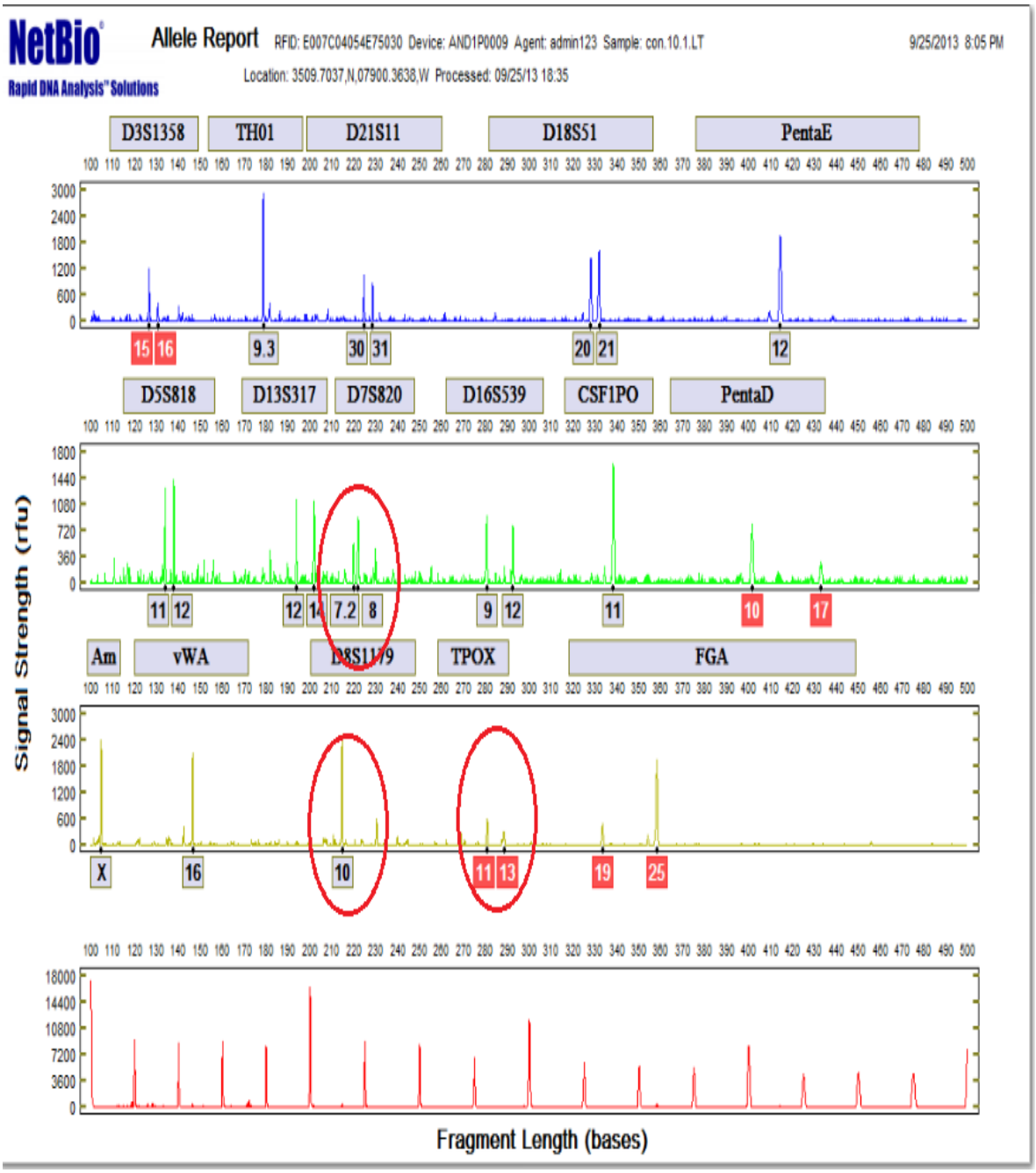
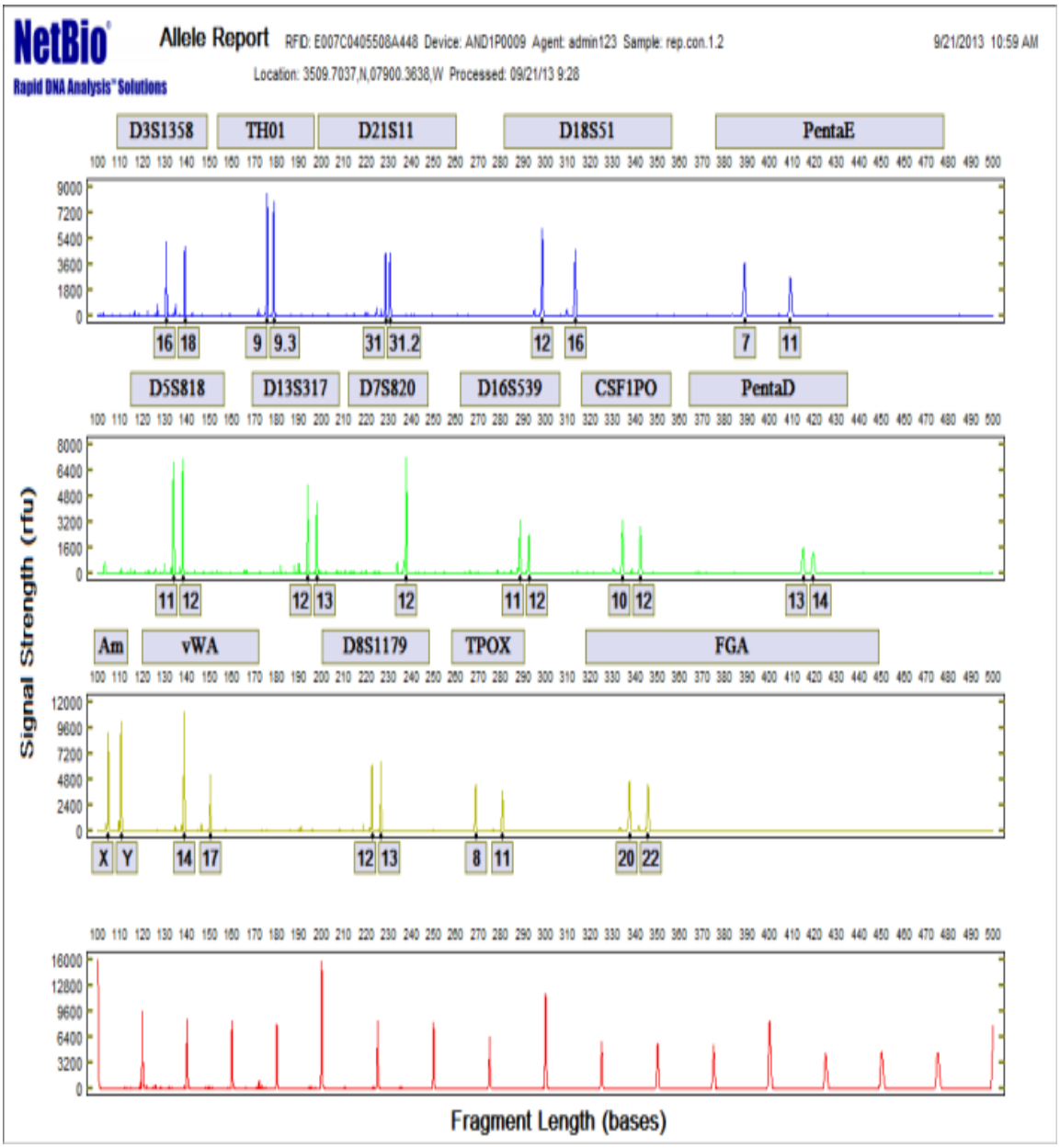


Figure11: An electropherogram generated from a lightly collected sample. The circled loci indicate loci where drop-out occurred. The 10 allele at the D7S820 locus dropped out; leaving only an 8 allele and an artifact that was mislabeled as a 7.2 allele. The 8 allele at the TPOX locus dropped out; leaving only an 11 allele and an artifact that was mislabeled as a 13 allele. Additionally, the 14 allele at the D8S1179 locus dropped out, leaving only the 10 allele.

Reproducibility

Twenty buccal swabs were collected from two different individuals. Each individual was swabbed ten separate times, and the swabs were run on the DNAscan instrument with each run consisting only of swabs that belonged to one individual. The profiles generated from the ten swabs collected from the first individual were compared to each other to determine whether there were any differences among them. The results showed that the profiles were comparable, and that the same alleles were called in each of these profiles (Figure 12a). Similarly, the profiles generated from the ten swabs collected from the second individual were compared to determine whether there were any differences among them. The results showed that the profiles were comparable and that the same alleles were called in all profiles attributed to an individual (Figure 12b). In one of the reproducibility runs, the allelic ladder present on the BSCS failed, and the pre-installed allelic ladder present in the instrument was employed to designate the allele calls. The alleles were all appropriately designated, and the final profiles were all generated successfully.

(a1)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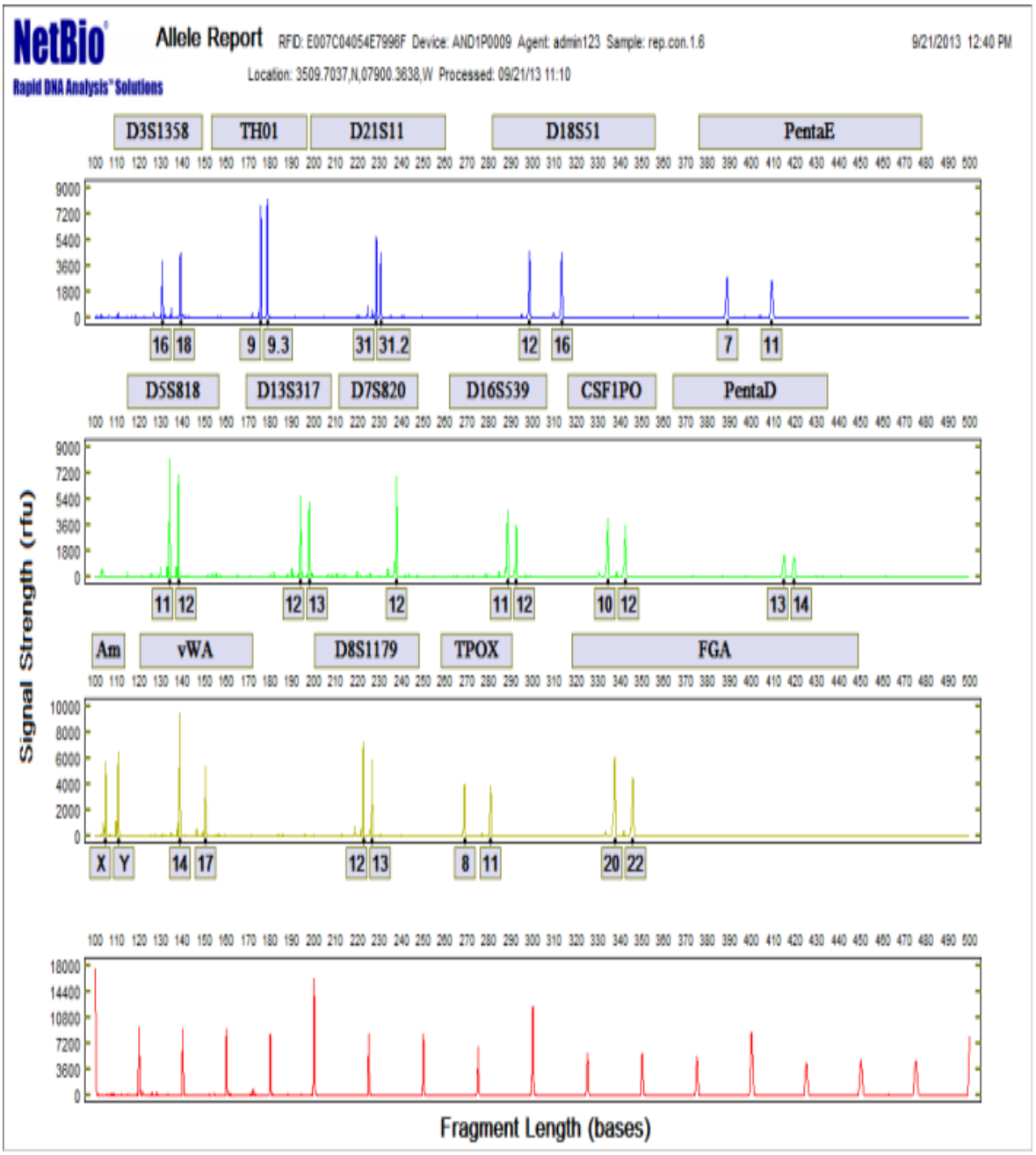
D18S51
PentaE
16 18
9 9.3
31 31.2
12 16
7 11
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
11 12
12 13
12
11 12
10 12
13 14
Am
vWA
D8S1179
TPOX
FGA
X Y
14 17
12 13
8 11
20 22
Signal Strength (rfu)
Fragment Length (bases)

(a2)


NetBio
Rapid DNA Analysis® Solutions
Allele Report
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D18S51
PentaE
16 18
9 9.3
31 31.2
12 16
7 11
D5S818
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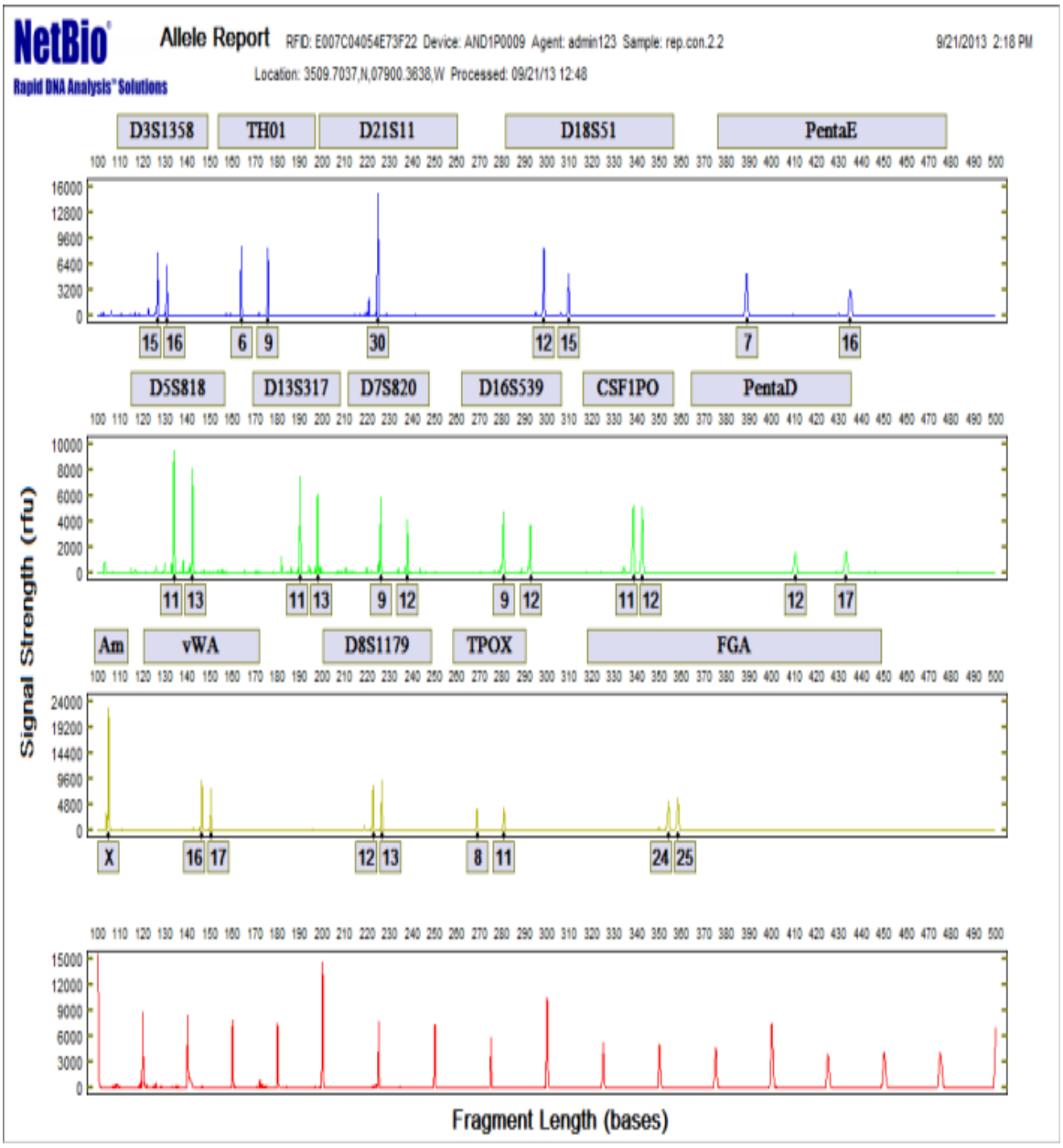
D7S820
D16S539
CSF1PO
PentaD
11 12
12 13
12
11 12
10 12
13 14
Am
vWA
D8S1179
TPOX
FGA
X Y
14 17
12 13
8 11
20 22
Signal Strength (rfu)
Fragment Length (bases)

(b1)


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
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9/21/2013 2:18 PM
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D18S51
PentaE
15 16
6 9
30
12 15
7
16
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
11 13
11 13
9 12
9 12
11 12
12
17
Am
vWA
D8S1179
TPOX
FGA
X
16 17
12 13
8 11
24 25
Signal Strength (rfu)
Fragment Length (bases)

(b2)

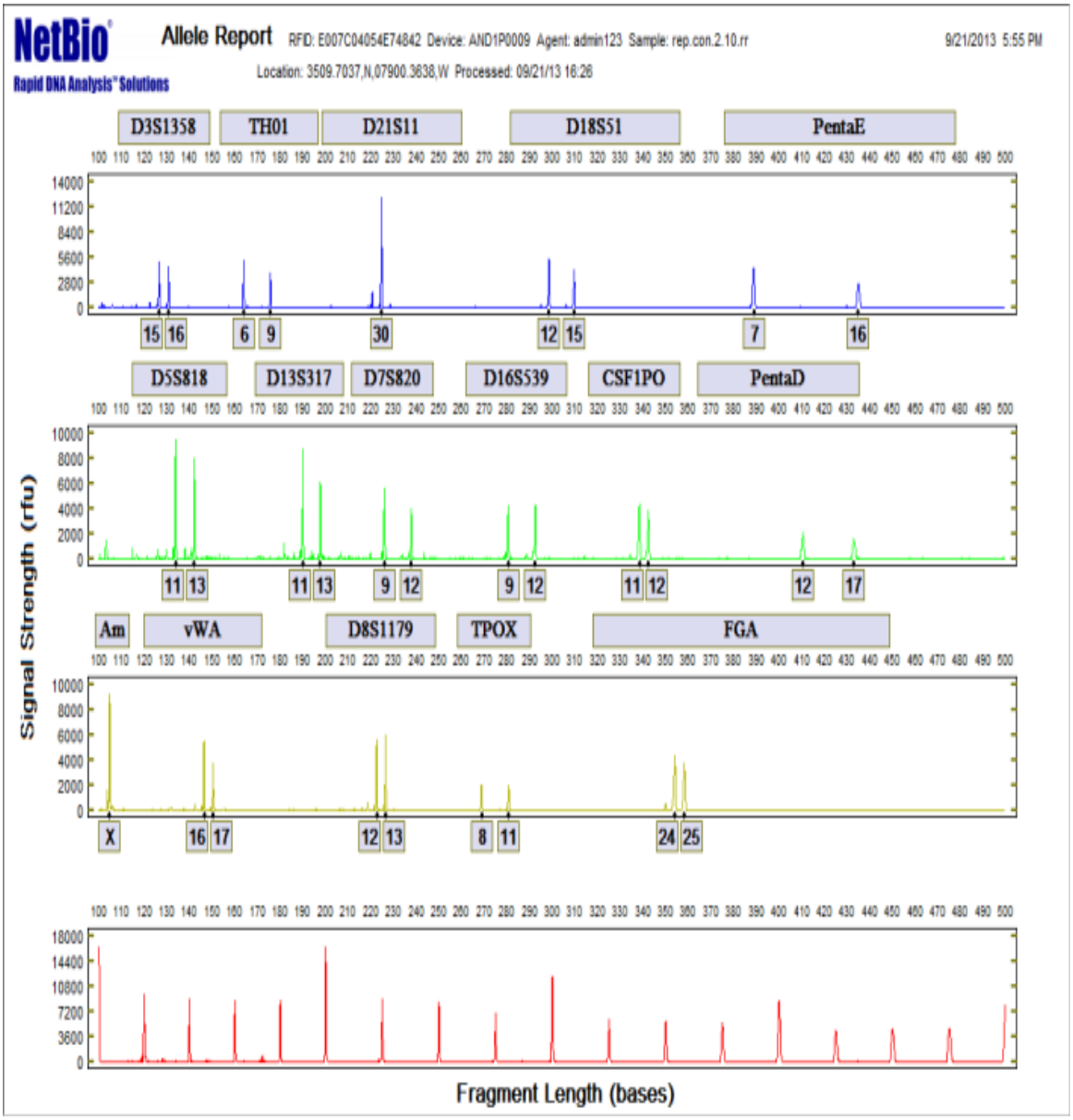
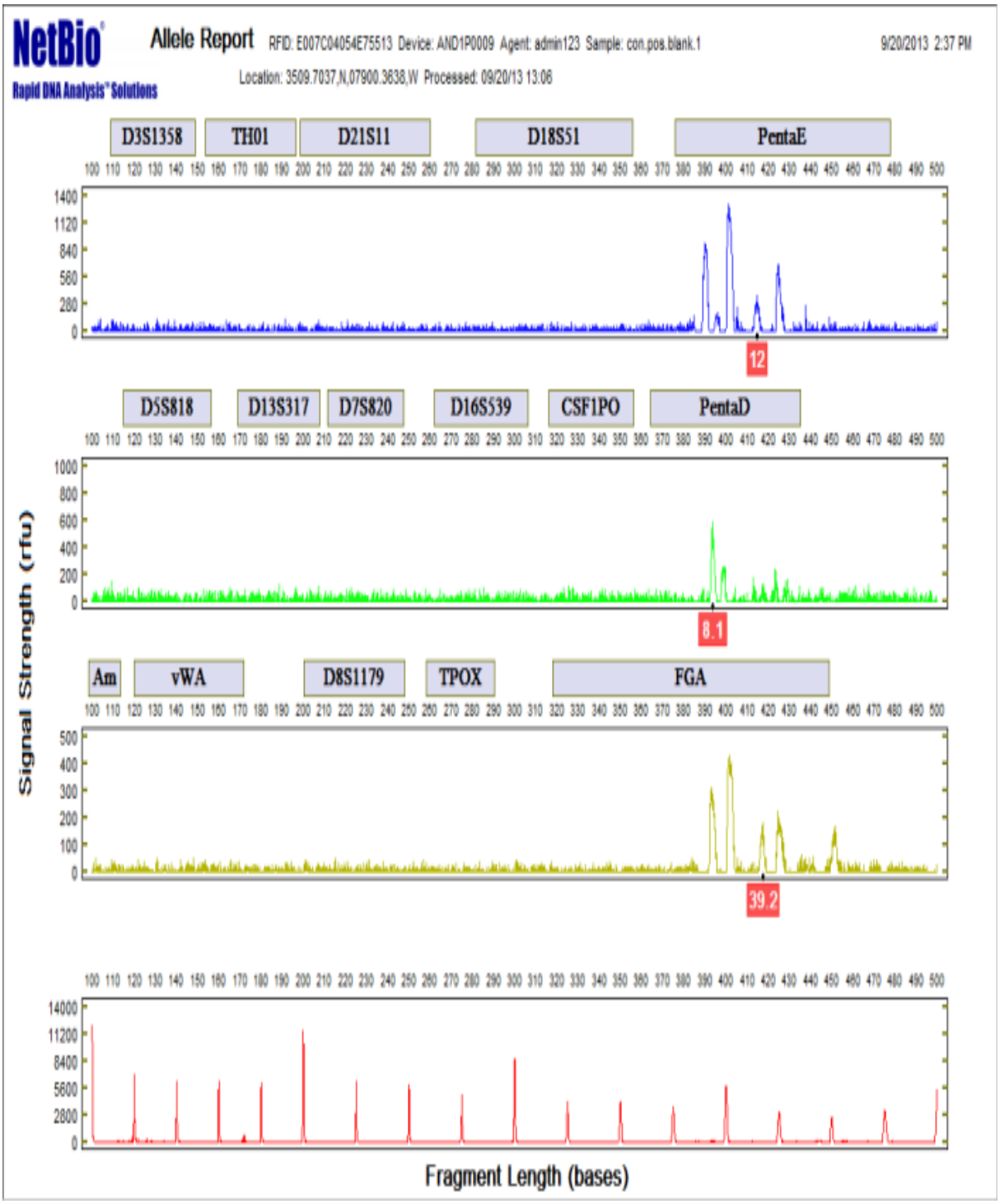


Figure 12: Example of two profiles generated from swabs obtained from the same individual, each of which was produced during separate runs on the DNAscan instrument (a1-2). Another example of two profiles generated from swabs obtained from the same individual. Each profile was produced during separate runs on the DNAscan instrument (b1-2).

Contamination

A contamination study was conducted by collecting ten buccal swabs from one individual to serve as known positive controls. These samples were run in conjunction with blank swabs that served as negative controls. As expected, the positive controls from each run all generated the same profile; however, there were a few anomalies observed in the blank profiles generated from the negative controls in these runs. In one run, two of the negative controls resulted in blank profiles that contained some regions with peaks (Figure 13). Additionally, in another negative control whose profile was generated in a separate run, there appeared to be some allelic ladder intra-run contamination (Figure 14). However, despite the spurious artifacts and allelic ladder contamination seen in these few negative controls, the profiles generated from the positive control swabs remained unaffected. It is important to point out that should these events have occurred in profiles generated from reference samples, they are readily detected by the software as well as by the user.


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
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9/20/2013 2:37 PM
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12
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D13S317
D7S820
D16S539
CSF1PO
PentaD
8.1
Am
vWA
D8S1179
TPOX
FGA
39.2
Signal Strength (rfu)
Fragment Length (bases)

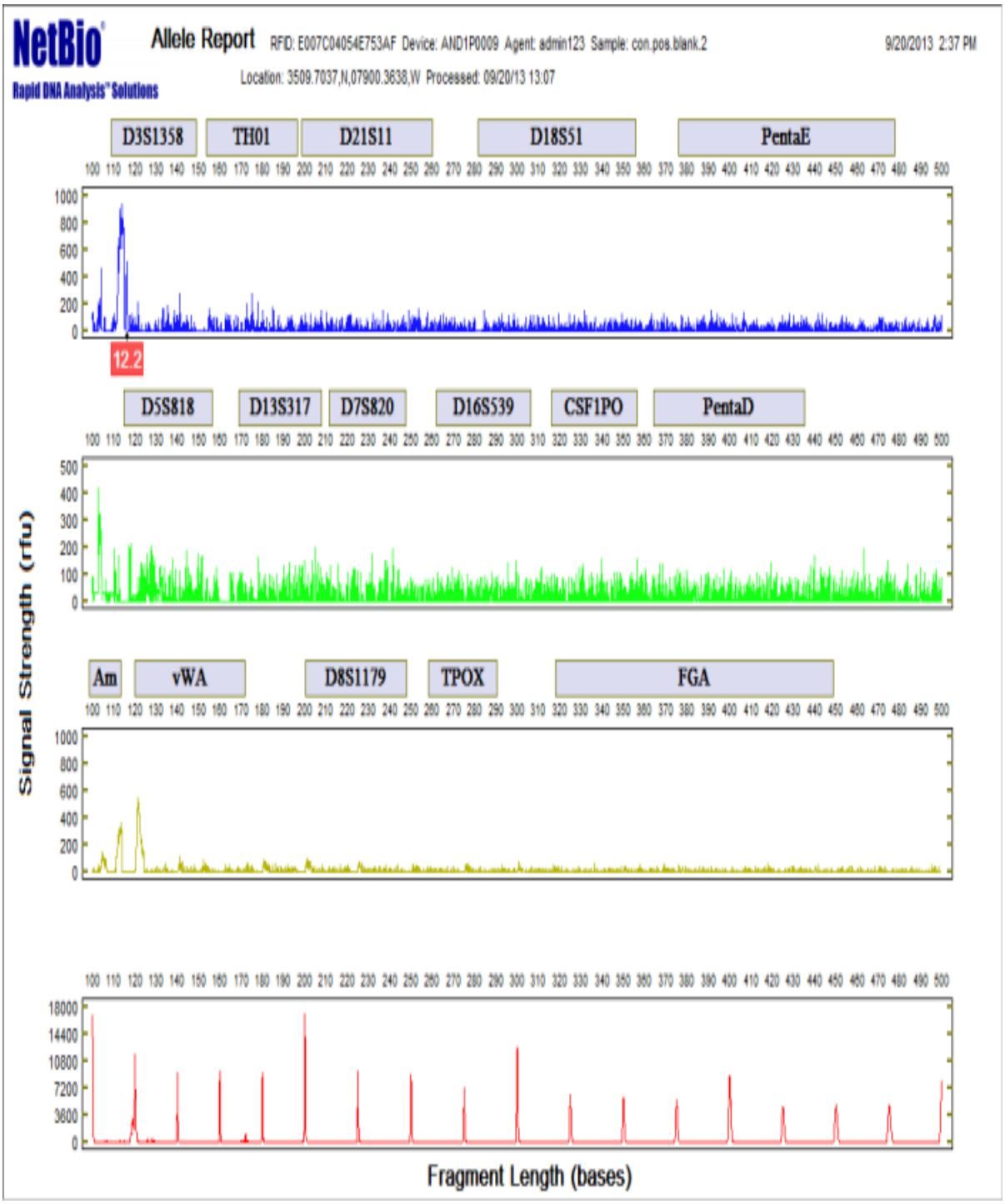


Figure 13: Electropherograms from two negative control blank profiles from the same run (top and bottom). Relatively high level peaks can be seen in each profile.

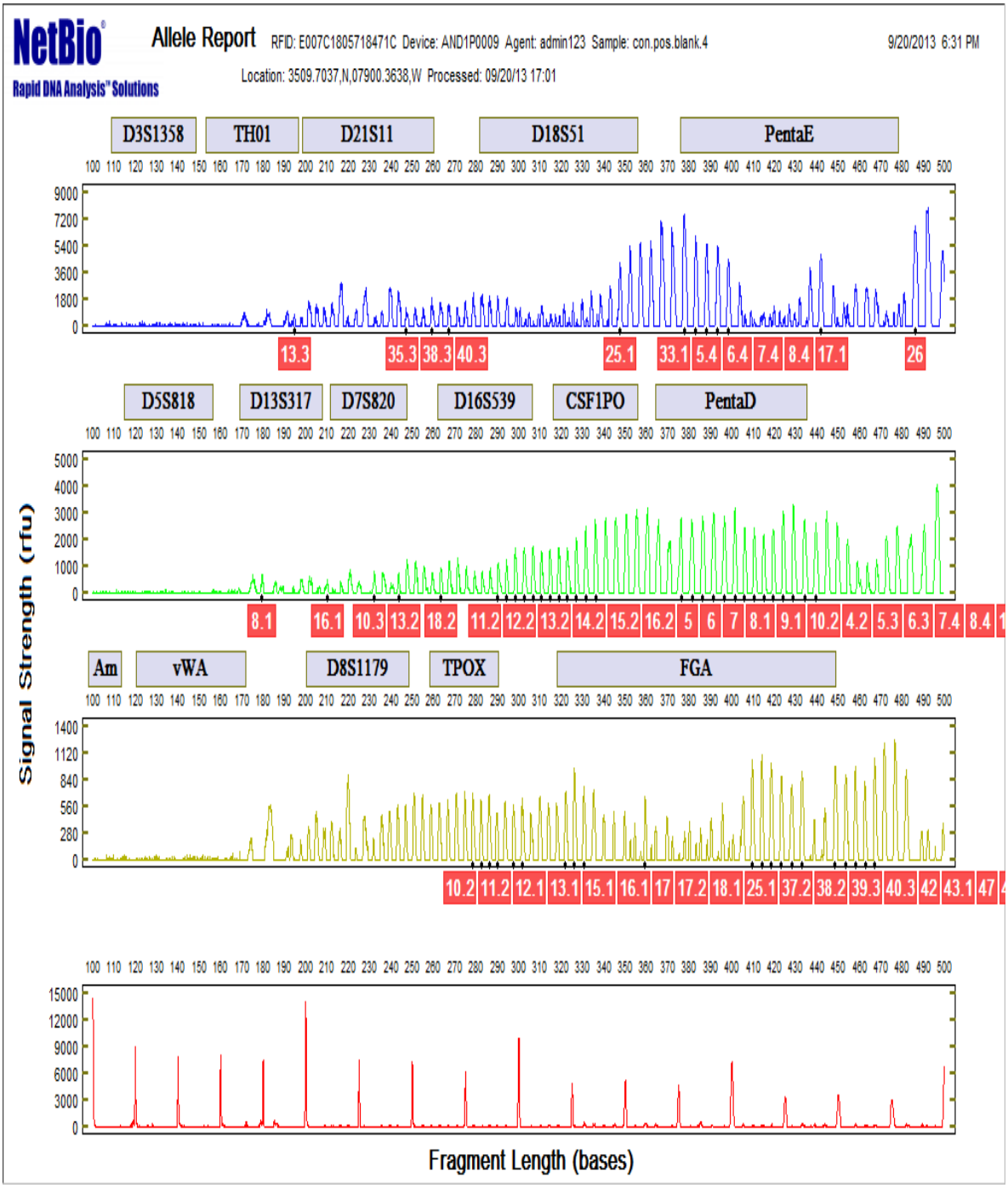


Figure 14: Electropherogram from of a negative control obtained from a blank swab. Evidence of intra-run allelic ladder contamination can be seen.

Inhibition

To test the effect of recent food or beverage consumption on STR profiles generated on the DNAscan instrument, buccal swabs were collected from individuals immediately following consumption of food or beverage. The resulting profiles did not show any evidence of inhibition (Figure 15). The peak heights were all well above the detection and stochastic thresholds of 150 RFU and 250 RFU respectively, and the peak height ratios were all above the 0.37 ratio, below which the instrument software flags the alleles as imbalanced.

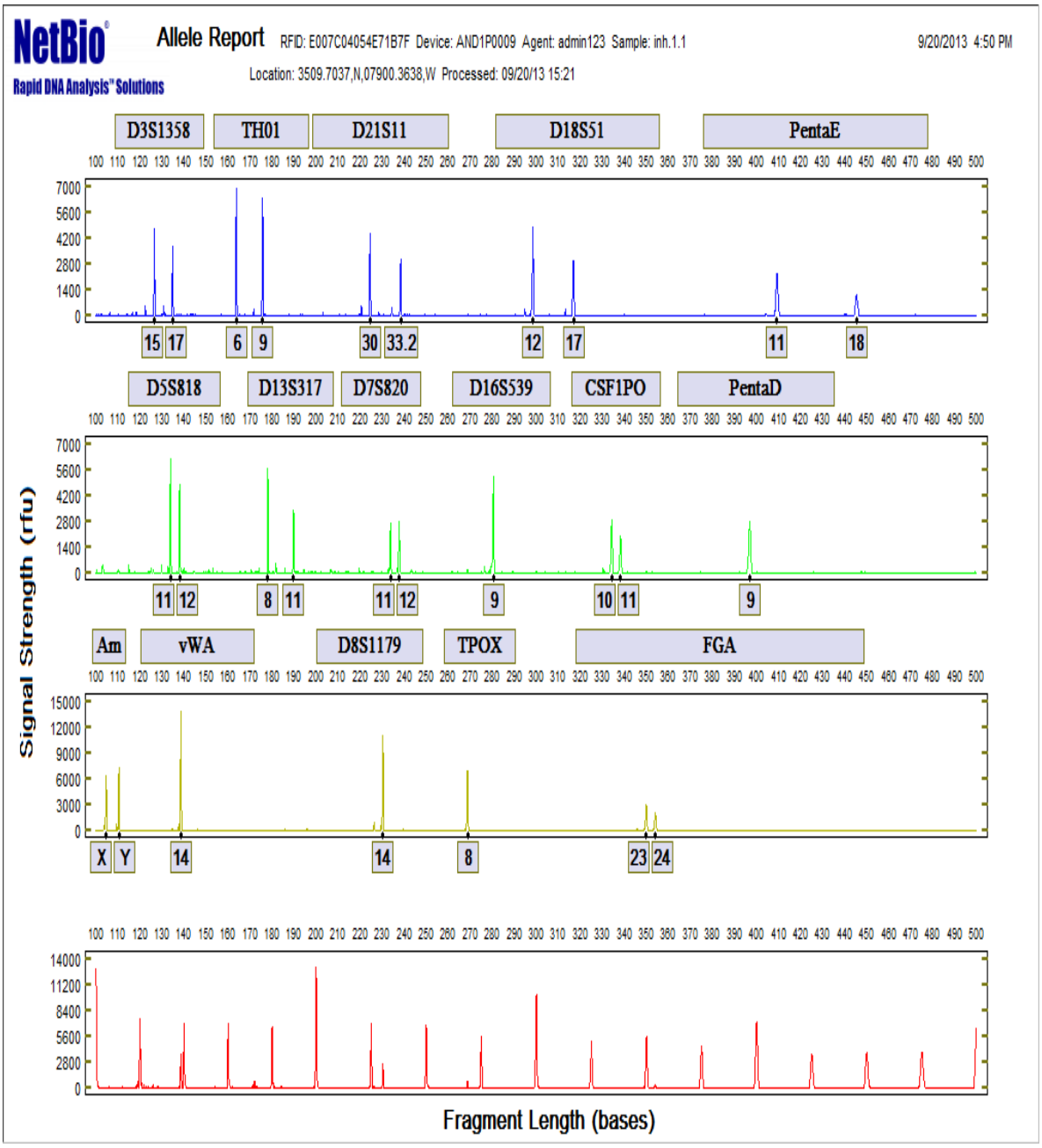


Figure 15: An electropherogram generated during the inhibition study performed on the DNAscan instrument.

Concordance

To test whether profiles generated using the DNAscan instrument matched those that were generated using standard STR typing techniques; a concordance study was performed. The results showed that all of the profiles generated on the DNAscan were concordant with those generated using bench-top STR typing methods (Figure 16). Two of these profiles (12.50%) were partial profiles (Table 1). One sample failed to yield a result with the standard STR typing method (Table 2). The Amelogenin marker on one sample failed to show the presence of a Y chromosome-specific PCR product in the profile generated on the DNAscan. This same sample only showed a nominal peak at this location in the profile generated using standard DNA typing methods (Figure 17). This consistent occurrence is most likely due to an incompatible primer binding site or a deletion on the Y chromosome, leading to a failure to amplify this region of interest. These Y results are concordant and are inherent in the individual.

Table 1: Overview of concordance study results.

	Total samples	Concordant profiles	Discordant profiles	Full Profiles	Partial Profiles
Sample Number (Percent)	16 (100.00%)	16 (100.00%)	0 (0.00%)	14 (87.5%)	2 (12.50%)

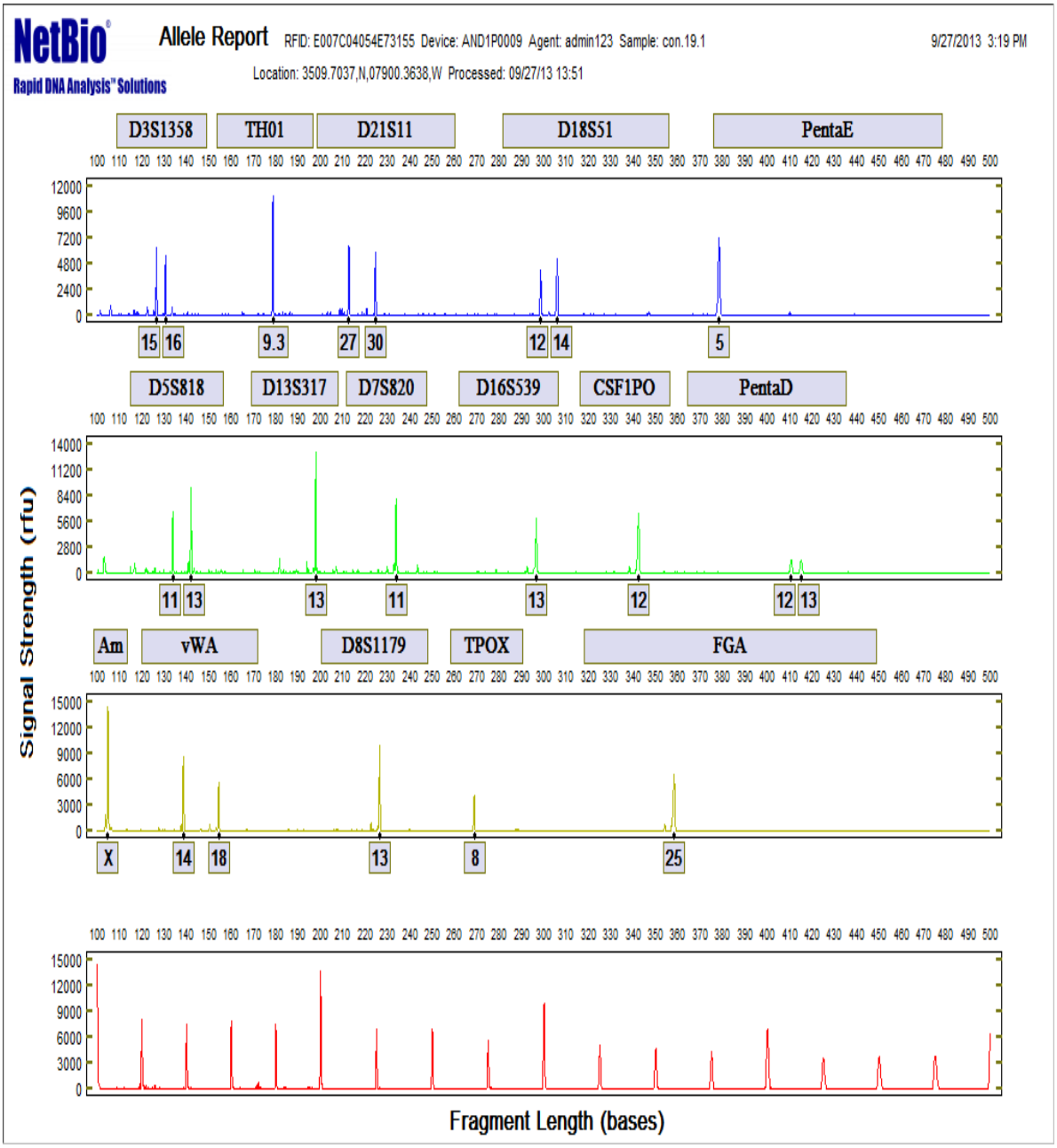
Table 2: Comparison of Concordance Study Profiles. † One of both methods failed to generate a profile. *Allele drop-out.

Sample	STR Typing Method	Locus							
		D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820
Con.1	DNAscan™	15, 17	6, 7	30, 32	13, 15	10, 19	9, 11	8, 13	9, 10
	Bench-top	15, 17	6, 7	30, 32	13, 15	10, 19	9, 11	8, 13	9, 10
Con.2	DNAscan™	15, 17	6, 6	29, 33.2	16, 17	10, 12	11, 12	8, 9	10, 12
	Bench-top	15, 17	6, 6	29, 33.2	16, 17	10, 12	11, 12	8, 9	10, 12
Con.3	DNAscan™	16, 16	7, 8	28, 28	16, 17	5, 12	12, 14	12, 12	10, 11
	Bench-top	16, 16	7, 8	28, 28	16, 17	5, 12	12, 14	12, 12	10, 11
Con.4†	DNAscan™	17, 18	7, 7	28. 30	13, 20	7. 10	12, 14	11, 12	11, 11
	Bench-top								
Con.5	DNAscan™	16, 17	7, 9.3	30.2, 32.2	12, 13	11, 19	11, 12	9, 11	11, 12
	Bench-top	16, 17	7, 9.3	30.2, 32.2	12, 13	11, 19	11, 12	9, 11	11, 12
Con.6†	DNAscan™								
	Bench-top	13, 15	9, 9.3	29, 30	16, 17	7, 12	12, 13	11, 11	8, 11
Con.7	DNAscan™	16, 17	6, 6	30, 32.2	13, 16	5, 11	12, 13	9, 11	7, 8
	Bench-top	16, 17	6, 6	30, 32.2	13, 16	5, 11	12, 13	9, 11	7, 8
Con.8	DNAscan™	15, 15	6, 8	30, 32.2	14, 20	14, 17	11, 11	10, 12	9, 9
	Bench-top	15, 15	6, 8	30, 32.2	14, 20	14, 17	11, 11	10, 12	9, 9
Con.9	DNAscan™	15, 17	9.3, 9.3	30, 31	15, 15	7, 14	11, 12	11, 12	9, 11
	Bench-top	15, 17	9.3, 9.3	30, 31	15, 15	7, 14	11, 12	11, 12	9, 11
Con.10	DNAscan™	*, *	9.3, 9.3	*, 31	*, *	12, 12	*, 12	*, 14	8, *
	Bench-top	15, 16	9.3, 9.3	30, 31	20, 21	12, 12	11, 12	12, 14	8, 10
Con.12	DNAscan™	16, 16	8, 8	28, 32.2	17, 17	10, 12	11, 12	9, 10	11, 13
	Bench-top	16, 16	8, 8	28, 32.2	17, 17	10, 12	11, 12	9, 10	11, 13
Con.13	DNAscan™	16, 16	6, 7	29, 31	16, 19	10, 16	10, 14	10, 12	11, 12
	Bench-top	16, 16	6, 7	29, 31	16, 19	10, 16	10, 14	10, 12	11, 12

Con.15	DNAscan™	15, 16	9.3, 9.3	29, 31	12, 13	10, 14	10, 12	11, 11	8, 9
	Bench-top	15, 16	9.3, 9.3	29, 31	12, 13	10, 14	10, 12	11, 11	8, 9
Con.16	DNAscan™	15, 16	7, 9.3	28, 34.2	13, 19	12, 13	11, 12	10, 14	10, 12
	Bench-top	15, 16	7, 9.3	28, 34.2	13, 19	12, 13	11, 12	10, 14	10, 12
Con.17	DNAscan™	16, 17	6, 9	29, 31	13, 16	12, 23	10, 11	9, 14	10, 11
	Bench-top	16, 17	6, 9	29, 31	13, 16	12, 23	10, 11	9, 14	10, 11
Con.18	DNAscan™	17, 18	6, 9.3	27, 31.2	10, 16	12, 15	11, 12	11, 14	8, 12
	Bench-top	17, 18	6, 9.3	27, 31.2	10, 16	12, 15	11, 12	11, 14	8, 12
Con.19	DNAscan™	15, 16	9.3, 9.3	27, 30	12, 14	5, 5	11, 13	13, 13	11, 11
	Bench-top	15, 16	9.3, 9.3	27, 30	12, 14	5, 5	11, 13	13, 13	11, 11
Con.20	DNAscan™	16, 16	6, 9.3	30, 32.2	17, 21	7, 12	9, 13	12, 12	9, 13
	Bench-top	16, 16	6, 9.3	30, 32.2	17, 21	7, 12	9, 13	12, 12	9, 13

Sample	STR Typing Method	Locus							
		D16S539	CSF1PO	Penta D	Amel	vWA	D8S1179	TPOX	FGA
Con.1	DNAscan™	10, 11	10, 12	11, 13	X, X	17, 18	13, 14	9, 11	20, 20
	Bench-top	10, 11	10, 12	11, 13	X, X	17, 18	13, 14	9, 11	20, 20
Con.2	DNAscan™	11, 11	10, 12	13, 14	X, X	14, 16	16, 17	8, 11	19, 23
	Bench-top	11, 11	10, 12	13, 14	X, X	14, 16	16, 17	8, 11	19, 23
Con.3	DNAscan™	10, 11	11, 12	9, 10	X, X	14, 16	14, 14	10, 11	25, 25
	Bench-top	10, 11	11, 12	9, 10	X, X	14, 16	14, 14	10, 11	25, 25
Con.4†	DNAscan™	12, 12	10, 12	11, 12	X, X	15, 16	9, 15	8, 8	20, 26
	Bench-top								
Con.5	DNAscan™	10, 10	12, 12	10, 10	X, X	16, 19	12, 16	9, 12	20, 25
	Bench-top	10, 10	12, 12	10, 10	X, X	16, 19	12, 16	9, 12	20, 25
Con.6†	DNAscan™								
	Bench-top	11, 12	10, 12	12, 13	X, X	14, 17	10, 11	8, 11	20, 20
Con.7	DNAscan™	11, 12	10, 12	9, 14	X, Y	14, 16	11, 14	8, 12	21, 25

	Bench-top	11, 12	10, 12	9, 14	X, Y	14, 16	11, 14	8, 12	21, 25
Con.8	DNAscan™	9, 12	12, 12	9, 12	X, X	14, 17	12, 14	8, 11	19, 21
	Bench-top	9, 12	12, 12	9, 12	X, X	14, 17	12, 14	8, 11	19, 21
Con.9	DNAscan™	12, 12	11, 12	10, 11	X, X	17, 18	13, 14	8, 9	22, 27
	Bench-top	12, 12	11, 12	10, 11	X, X	17, 18	13, 14	8, 9	22, 27
Con.10	DNAscan™	9, 12	11, 11	10, *	*, *	*, *	*, *	*, *	19, 25
	Bench-top	9, 12	11, 11	10, 17	X, X	16, 16	10, 14	8, 11	19, 25
Con.12	DNAscan™	11, 13	11, 12	12, *	X, Y	17, 18	13, 15	11, 11	21.2, 22
	Bench-top	11, 13	11, 12	12, 13	X, Y	17, 18	13, 15	11, 11	21.2, 22
Con.13	DNAscan™	11, 12	12, 13	9, 11	X, Y	14, 14	13, 15	8, 11	19, 23
	Bench-top	11, 12	12, 13	9, 11	X, Y	14, 14	13, 15	8, 11	19, 23
Con.15	DNAscan™	11, 12	12, 12	9, 13	X, Y	15, 15	9, 9	8, 8	20, 21
	Bench-top	11, 12	12, 12	9, 13	X, Y	15, 15	9, 9	8, 8	20, 21
Con.16	DNAscan™	11, 12	11, 11	10, 12	X, *	14, 18	10, 13	8, 8	22, 25
	Bench-top	11, 12	11, 11	10, 12	X, Y	14, 18	10, 13	8, 8	22, 25
Con.17	DNAscan™	12, 13	11, 12	9, 13	X, Y	17, 18	13, 14	8, 8	20, 23
	Bench-top	12, 13	11, 12	9, 13	X, Y	17, 18	13, 14	8, 8	20, 23
Con.18	DNAscan™	11, 12	11, 12	9, 12	X, X	16, 18	10, 13	8, 8	19, 23
	Bench-top	11, 12	11, 12	9, 12	X, X	16, 18	10, 13	8, 8	19, 23
Con.19	DNAscan™	13, 13	12, 12	12, 13	X, X	14, 18	13, 13	8, 8	25, 25
	Bench-top	13, 13	12, 12	12, 13	X, X	14, 18	13, 13	8, 8	25, 25
Con.20	DNAscan™	11, 13	12, 13	12, 12	X, X	17, 17	12, 13	8, 8	23, 24
	Bench-top	11, 13	12, 13	12, 12	X, X	17, 17	12, 13	8, 8	23, 24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Rapid DNA Analysis™ Solutions
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FGA
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25
Signal Strength (rfu)
Fragment Length (bases)

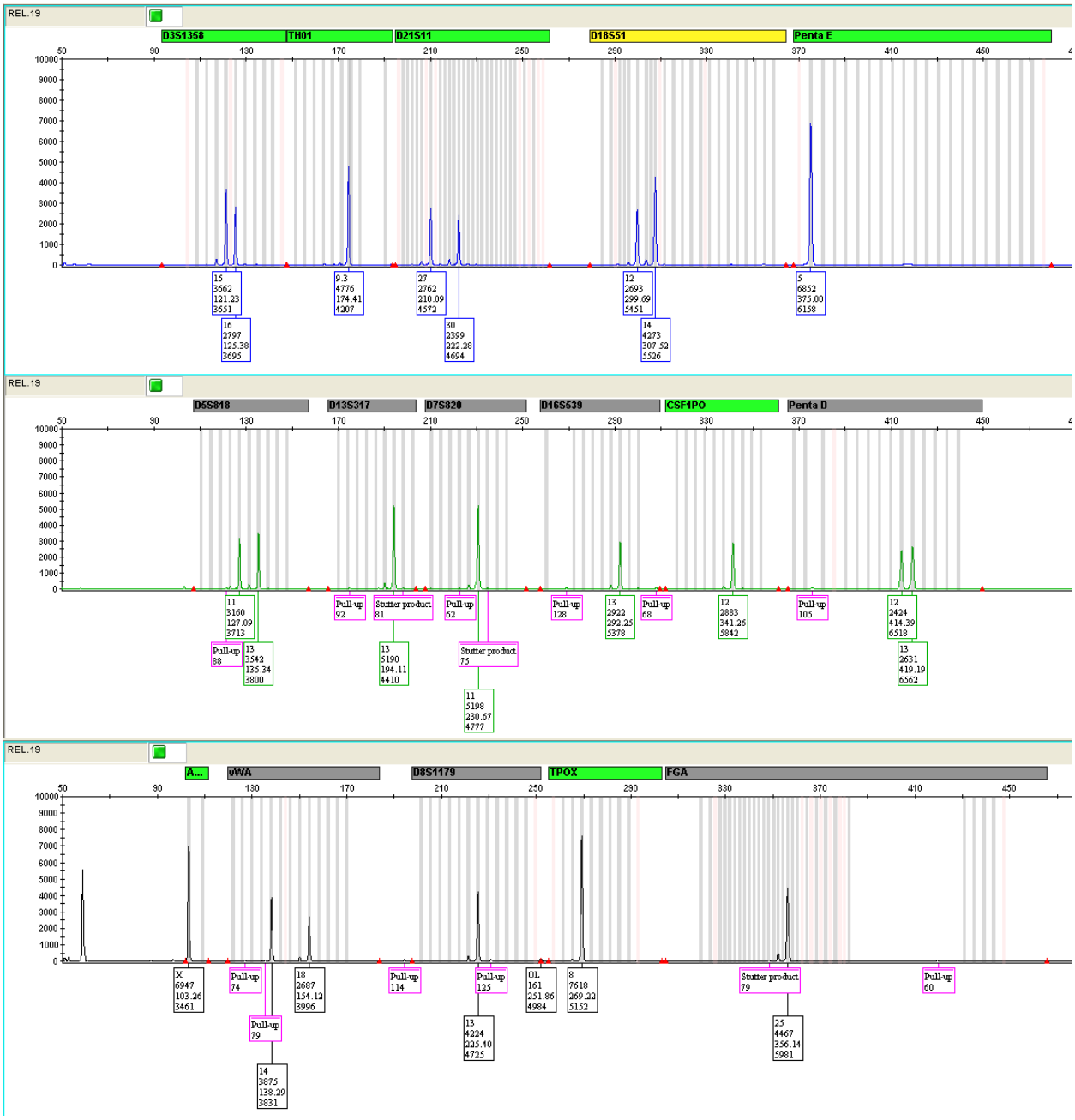
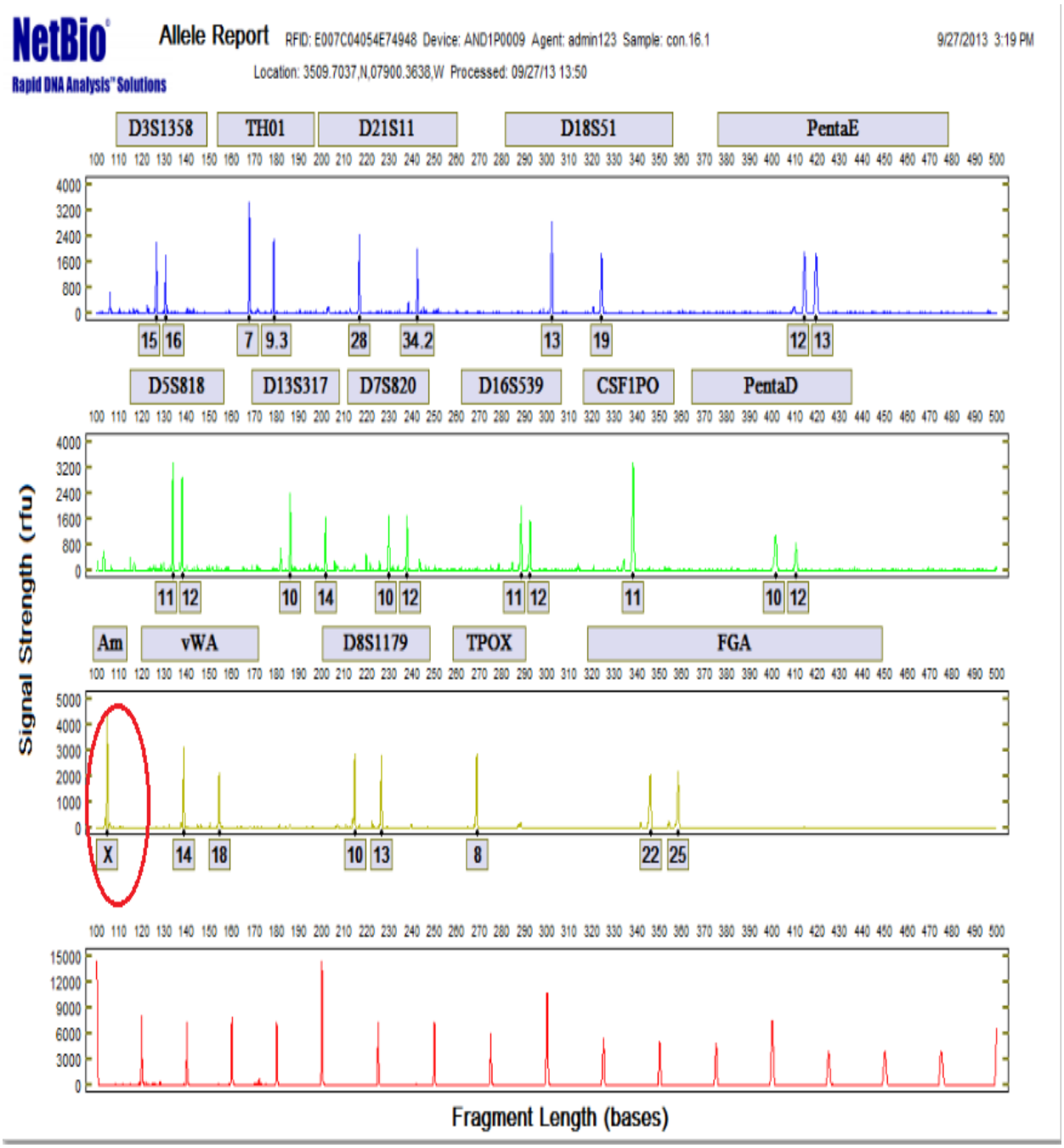


Figure 16: An example of two profiles showing that they are comparable to one another. The first profile (top) was generated on the DNAscan while the second (bottom) was generated using a standard DNA typing method.


NetBio
Rapid DNA Analysis™ Solutions
Allele Report
RFID: E007C04054E74948 Device: AND1P0009 Agent: admin123 Sample: con.16.1
9/27/2013 3:19 PM
Location: 3509.7037,N,07900.3638,W Processed: 09/27/13 13:50
D3S1358
TH01
D21S11
D18S51
PentaE
15 16
7 9.3
28
34.2
13
19
12 13
D5S818
D13S317
D7S820
D16S539
CSF1PO
PentaD
11 12
10
14
10 12
11 12
11
10 12
Am
vWA
D8S1179
TPOX
FGA
X
14
18
10 13
8
22 25
Signal Strength (rfu)
Fragment Length (bases)

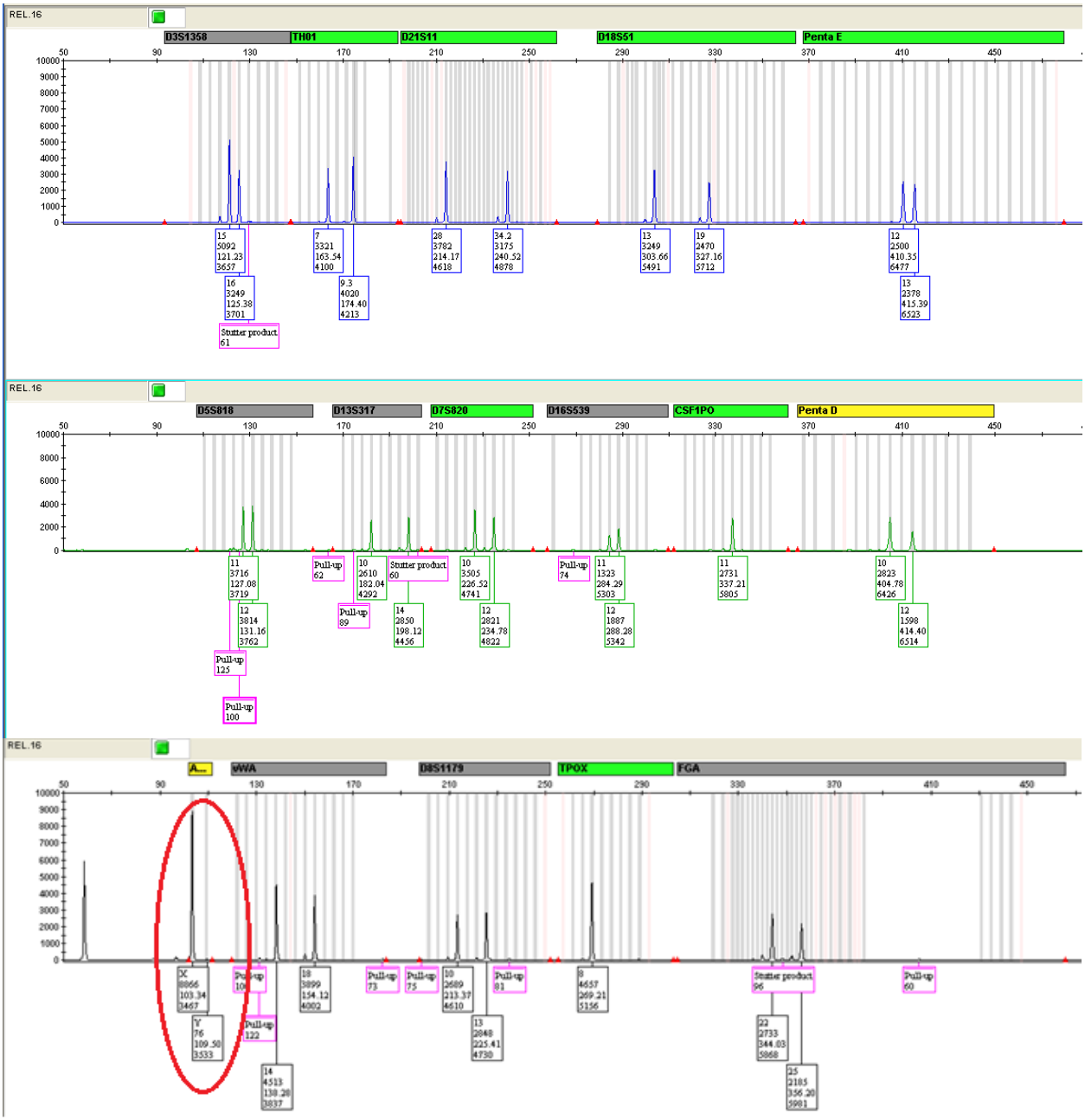


Figure 17: Electropherograms showing a sample that failed to properly generate the Y-specific PCR product. The top image is the electropherogram generated on the DNAscan. The bottom electropherogram was generated using standard STR tying methods. These results are concordant and inherent to the sample.

CHAPTER 4

DISCUSSION

The purpose of this project was to test the hypothesis that automated rapid STR genotyping platforms perform as well as standard STR typing methods for typing reference samples by comparing the ability of NetBio's DNAscan automated rapid STR genotyping platform and standard STR typing methods to generate DNA profiles from reference samples. Sensitivity studies were conducted with the collection and DNA typing of twenty heavily collected samples as well as twenty lightly collected samples, all of which exhibited good peak morphology and yielded profiles which were similar to those generated using standard STR typing methods. Allele drop-out was observed in only one of the lightly collected samples as well as two of the heavily collected samples, and one heavily collected sample failed to generate a profile on the DNAscan instrument (Table 3). Peak height imbalance was not observed in any of the heavily collected samples and was seen in only one of the lightly collected samples (Table 3). However, in all of these samples, the loci in question were flagged or called inconclusive by the DNAscan software, reducing the risk of mistyping the profiles. Overall, both buccal swab collection techniques performed similarly to one another in this study. The variation seen between profiles generated with each collection method can be explained by the fact that while the collection techniques were consistent, some individuals shed more epithelial cells than others, regardless of the collection method.

Table 3: Summary of sensitivity studies performed on the DNAscan.

Collection Method	Total Sample Number	Samples With Peak Height Imbalance	Full Profiles	Partial Profiles	Samples With No Profile	Percent of Buccal Swabs With Partial Profiles	Percent of Buccal Swabs With Full Profiles	Percent of Buccal Swabs With No Profile
Heavy	20	0	17	2	1	10.00%	85.00%	5.00%
Light	20	1	19	1	0	5.00%	95.00%	0.00%

Reproducibility studies demonstrated that the DNAscan automated rapid STR typing system was capable of consistently generating identical profiles from the same individual. A total of four runs were completed using twenty swabs, ten of which were obtained from one individual while the other ten were obtained from a different individual. For all ten swabs obtained from the same individual, 100 percent of the alleles were identically labeled. The failure of the allelic ladder present on the BCSC used for one of the runs demonstrated the ability of this instrument to use its onboard allelic ladder to designate alleles properly in the event of such a failure.

Contamination studies showed no indication of inter-run contamination, and none of the positive controls exhibited evidence of contamination occurring. While two of the negative controls from one run showed extra peaks, these events did not affect the profiles generated from the positive controls in those runs. Intra-run allelic ladder contamination was seen in only one negative control sample; however, the profiles generated in the run were still correctly called.

Inhibition studies performed with buccal swabs collected after consumption of food or beverage yielded profiles that showed no evidence of inhibition. In the profiles produced from the samples used for this part of the study, 100 percent of the expected alleles were present,

indicating that there was no allele drop-out as a result of the recent food and/or beverage consumption prior to buccal swab collection. In real-life settings, the activities of an individual prior to buccal swab collection cannot always be controlled; therefore, the ability of this technology to yield full profiles after eating or drinking is a good indication of the robustness of the system.

As seen in Table 1, when concordance study samples were performed, 100 percent of the profiles generated on the DNAscan instrument were concordant with those that were generated using a standard STR typing method. While there were two samples that only generated partial profiles on the DNAscan, the alleles that were present within these profiles were concordant with the alleles seen in the profiles produced with the bench-top method. In one sample, PCR amplification of the Amelogenin marker failed to show a Y-specific product for both typing methods. This event was concordant between the two analytical approaches and most likely is due to inability of the primers to anneal to their binding site, leading to failed amplification of this chromosome-specific region.

CHAPTER 5

CONCLUSION

In conclusion, the results of this project demonstrated that the automated rapid STR typing method was capable of generating quality STR profiles, often similar to that of standard STR typing. While there was slightly more peak height imbalance and allele drop-out seen in the heavily collected buccal swabs, this fully-automated, rapid DNA typing instrument was capable of producing full profiles regardless of the collection methods used when collecting buccal swabs. Overall, the data indicated that full profiles could be generated with 85 percent of heavily collected buccal swabs and 95 percent of lightly collected buccal swabs on the DNAscan (Table 3).

The results obtained in the reproducibility experiments appeared to indicate that buccal swabs obtained from the same individual could be run in different swab chambers on the BCSC as well as on completely separate BCSCs and consistently generate the same DNA profile. This study also demonstrated the ability of the instrument software to appropriately designate allele calls through the use of the on-board allelic ladder in the event that the pre-loaded allelic ladder on the BCSC failed. All of the profiles generated during this study were consistent with one another, even when the pre-loaded allelic ladder failed.

There was no contamination detected in the inter-run testing. However, one run did show allelic ladder contamination. The most likely explanation for this observation is that the

reagents for the BCSCs used for this study currently are assembled manually. Thus, the allelic ladder cross-over was likely a manufacturing error. Despite this occurrence, all of the alleles in the positive control profiles were present and correctly called, and there were no spurious alleles observed.

The data obtained from the inhibition study indicated that daily activities such as eating food or drinking beverages do not have a significant effect on the STR profiles generated on the DNAscan. There was no evidence of peak height imbalance or decreased peak heights that would indicate possible inhibition. The finding that these actions do not seem to alter the quality of the profiles created is a good indication of robustness of the system. It is advantageous for all methods of STR typing to be capable of producing full DNA profiles regardless of these types of activities prior to sample collection.

Evaluation of the results of the concordance study revealed that the profiles generated using the DNAscan instrument were comparable with those obtained using a standard bench-top DNA typing technique. The failure of the Y-specific PCR product to amplify in one of the samples under both analytical streams further supports concordance.

There were a couple of limitations to this project. The first of these was that buccal swabs could not be recovered once they were inserted into the BCSC. The sample chambers are designed in such a way that the specially designed swabs click into place and cannot be removed without breaking the BCSC. While this is a desirable feature that helps to avoid possible sample switching and contamination, it does limit re-analysis of a sample if a run fails. However, this limitation can be overcome by collecting an additional buccal swab from each individual so that a back-up swab is available should a sample need to be re-processed. Another limitation in this

study was that if a pre-loaded allelic ladder fails, under standard interpretation protocols the data from that BCSC cannot be used. However, as was demonstrated with the failed ladder seen in this study, this limitation can be mitigated through the use of the on-board allelic ladder that is installed within the DNAscan instrument software. As was seen, this ladder allowed the successful generation of complete STR profiles, even after the failure of the BCSC's pre-loaded ladder.

Overall, this study successfully demonstrated the ability of this rapid DNA genotyping platform to generate profiles comparable with standard STR typing methods for typing reference samples. The DNAscan system was shown to be reliable with regard to sensitivity, contamination, reproducibility, and inhibition, making it an attractive alternative to bench-top STR typing methods. Additionally, the STR profiles generated on the DNAscan were comparable with those generated using standard methods, with 85 to 95 percent of the reference samples processed on the DNAscan yielding profiles that were concordant with those generated using standard bench-top techniques. The data support the ability of this platform to perform DNA genotyping as well as more traditional methods.

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