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The BodePlex 3 multiplex is a sensitive mini-STR typing system for analyzing highly degraded and low copy number (LCN) DNA. It allows for the typing of smaller amplicons and decreased quantities of DNA template, thus enhancing the sensitivity of STR analysis of compromised samples. The present study validated BodePlex 3 multiplex system on the ABI PRISM® 3100 Genetic Analyzer to be used at The Bode Technology Group for forensic DNA analysis of small amplicons and LCN DNA. Validation experiments were performed according to the DNA Advisory Board (DAB) guidelines. Performance of the multiplex was accurate, reliable and reproducible. The results indicated that the typing system is highly specific for human DNA, sensitive for detecting profiles of LCN DNA, and is capable of resolving mixtures to a certain extent. In addition, this project outlined possible limitations that must be considered for successful use and interpretation of BodePlex 3 DNA profiling results.

VALIDATION OF BODEPLEX 3 FOR TYPING SMALL AMPLICONS AND LOW COPY NUMBER DNA

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INTERNSHIP PRACTICUM REPORT

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By

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LIST OF ABBREVIATIONS

ABI 3100, ABI PRISM® 3100 Genetic Analyzer

bp, base pair

BP3, BodePlex 3

CE, capillary electrophoresis

CODIS, COmbined DNA Index System

DAB, DNA Advisory Board

DNA, deoxyribonucleic acid

dNTP, deoxynucleotide triphosphate

FBI, Federal Bureau of Investigation

LCN, low copy number

OL, off-ladder allele

PCR, polymerase chain reaction

Pro/CO, Profiler Plus®/COfiler® PCR amplification kits

RFLP, restriction fragment length polymorphism

RFU, relative fluorescence unit

STR, short tandem repeat

TWGDAM, Technical Working Group on DNA Analysis Methods

VNTRs, variable number of tandem repeats

CHAPTER I

INTRODUCTION

Deoxyribonucleic acid (DNA) is the core element in the making of beings. In its structure lies the genetic code needed for directing the machinery of life. The human genome is composed of coding regions, also known as genes, and non-coding regions. DNA in coding regions contains the information required for the synthesis of proteins; it is transcribed and translated into cellular products essential for the functioning of cells. Only approximately 5 % of the human genome is made of genes (1); the remaining part is non-coding DNA i.e. it does not code for any products. The function of these areas is still being delineated. However, in forensics, they are assuming an increasingly important role. The markers that distinguish individuals and are used in human identity testing lie in these non-coding regions. Any two individuals share 99.9 % of their genome sequences (2). Variations in DNA sequences between individuals are termed polymorphisms. These variations lie in the remaining 0.1% of the genome sequence, in tandemly repeated DNA sequences that are dispersed throughout the human genome. A tandem repeat in DNA is two or more contiguous copies of a pattern of nucleotides. Tandem repeats were discovered in 1985 by Dr. Alec Jeffreys, who found that there are certain regions in the DNA sequence that are repeated over and over again next to each other, and that these

repeats differed in their number among individuals (1). They are typically classified into groups depending on the size of the repeat sequence. Minisatellites (variable number of tandem repeats, VNTRs) have repeats with 9-80 base pair (bp) units, while microsatellites (short tandem repeats, STRs) contain 2-7 bp repeats (3). Dr. Jeffreys developed the earliest technique used in human identity testing, one that examined the length variation of VNTRs. It involved the use of a restriction enzyme that cuts the flanking regions surrounding the repeat sequence and was called restriction fragment length polymorphism (RFLP) (1). After this breakthrough, DNA typing for human identity testing started its rise. Currently, the polymorphisms most widely used in forensic human identity testing are short tandem repeats (STRs). STRs have a significantly shorter length than VNTRs, usually between 100 and 450 bp, and their analysis requires a smaller amount of DNA template, which permits the examination of partially degraded DNA (4). These areas have shown such an extremely high degree of polymorphism among individuals in a population that they have become the genetic markers of choice in human identity testing.

Forensic DNA analysis relies on differences between individuals at unique genetic loci to determine whether two or more biological samples could have originated from the same source. These loci are examined by the polymerase chain reaction (PCR), a process that allows the replication of a specific DNA region repeatedly to yield millions of copies of the target sequence (5). PCR allows the concurrent amplification of multiple loci at a time, increasing the discrimination power of STR analysis. This technique is termed "multiplexing". Forensic applications of DNA testing include: crime scene

evidence, sexual assault evidence, paternity testing, missing persons investigations, identification of unknown soldiers, investigation of mass disasters, and to acquit wrongly accused people (4). The most common sources of DNA include: blood, semen, saliva, hair and bone. The condition of biological samples processed for forensic DNA testing is an important factor in the determination of the success of the analysis. DNA obtained from compromised samples is often excessively degraded or found in trace amounts not suitable for successful STR typing. Biological samples may be compromised by exposure to excessive heat, humidity, and environmental or chemical insults, all of which may lead to DNA degradation. In addition, some evidentiary samples, such as fingerprints and swabs of personal items, may have very low quantities of DNA. Examples of compromised biological samples include those obtained from a mass disaster investigation scene, such as those encountered during the analysis of samples recovered from the World Trade Centers. It has been previously reported that as the extent of genomic DNA degradation increases, loci fail to amplify in order of decreasing size, with the first loci to show reduced yield being the longest (6). Thus STR typing of these samples often results only in partial profiles and some fail to yield a profile at all. As the amount of starting DNA template decreases, generated profiles are distorted due to the effect of stochastic variations, causing allelic drop out, which leads to incorrect genotyping of a homozygous locus, and heterozygous peak imbalance. Allele drop in, or detection of "false" alleles, due to sporadic contamination has also been reported with analysis of trace DNA quantities (7). The typical amount of DNA template required for

successful STR typing is between 0.5 to 2 ng, depending on the specifications of commercially available STR kits.

The forensic community has been working towards the development of more sensitive techniques to deal with trace amounts of total genomic DNA, or amounts less than a 100 pg; termed low copy number (LCN) DNA (8). Advances in this area of research will allow for the examination of a wider range of evidentiary samples that cannot otherwise be analyzed with conventional STR typing, including compromised samples with degraded and trace amounts of DNA (ex: fingerprints, charred human remains, and minute amounts of evidentiary material). One method to enhance the generation of profiles from LCN templates is to increase the number of amplification cycles utilized. The conventional range of PCR cycle numbers used in STR analysis is 28-30 cycles (9). By increasing cycle number (for example up to 34 cycles), more amplification products (amplicons) are produced, and the amount of DNA template is increased. This approach is sensitive enough to detect a single molecule of DNA (10). However, this method has disadvantages including increases in the potential for allelic drop out due to stochastic variation which will preferentially amplify different alleles, increases in the degree of peak area asymmetry observed at heterozygous loci, and increases in the size range of stutter across loci due to sampling small DNA templates (8). In addition, as the number of PCR cycles is increased, there will be a higher incidence of laboratory-based contamination, even under stringent sterile conditions (10). Another approach to enhance sensitivity of STR analysis is nested PCR (11). For this technique, two sets of primers are used in two separate PCR reactions. The first reaction serves to

amplify the STR and adjacent regions. In the second round of PCR, an aliquot of the PCR product from the first reaction is used as a template, and is amplified with primers that are designed to target smaller amplicons. This technique reduces the amount of non-specific products and can analyze the DNA content of a single cell. One of the disadvantages of this method is the need to transfer the amplicons into a separate tube, thus increasing the incidence of contamination (10).

Following the challenges faced in analyzing thousands of samples from the World Trade Centers, The Bode Technology Group developed a robust and sensitive STR multiplex system, BodePlex 3, for analyzing highly degraded and LCN DNA. The multiplex examined the following STR loci: D3S1358, TH01, D5S818, D13S317, vWA, and D8S1179. Primers for this multiplex were chosen based on current data for the human genome at each STR locus, and were designed to target smaller DNA fragments, by bringing the complementary primer strands closer to the targeted repeat sequence. This approach allows for the typing of smaller amplicons and decreased quantities of DNA template, thus enhancing the sensitivity of STR analysis of compromised samples. The process of development of the system started with the evaluation of each potential primer sequence for formation of dimers, stability, hairpin formation and melting temperatures. Synthesized primers were then ordered from various vendors, with the forward primers being labeled with a fluorescent tag at the 5' end. The primers were tested in monoplexes at varying concentrations to determine the optimum primer concentration. Results were analyzed and the primer pairs were evaluated. Multiplexes of

the primer pairs were then tested with varying primer concentrations for all multiplex combinations to obtain the most balanced multiplex.

With the advent of forensic DNA typing, the Federal Bureau of Investigation (FBI) laboratory, together with several other forensic science laboratories, recognized the need for establishing guidelines to be followed by DNA testing laboratories. This would achieve standardization among the laboratories to assure the legal system and the public that DNA typing results conducted by a forensic laboratory are reliable, accurate and reproducible. In addition, these guidelines aimed to establish communication and concordance between DNA testing laboratories, with the ultimate goal being the development of a national DNA data bank, CODIS (COmbined DNA Index System). To meet this end, the Technical Working Group on DNA Analysis Methods (TWGDAM) was created in 1988 (12). Prior to implementation of an STR profiling system in a forensic laboratory, TWGDAM and the DNA Advisory Board (DAB) require that the system be validated according to their recommendations and quality assurance standards for forensic DNA testing laboratories. These standards place specific requirements on a DNA testing laboratory, with validation studies being one of these necessities. DAB quality assurance standards define validation as a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis. It consists of: (a) Developmental validation, which is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples, (b) Internal validation, or the accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected

in the laboratory (14). TWGDAM guidelines require that validation studies be conducted by the DNA laboratory prior to the adoption of a new procedure for forensic analysis (13). DAB standards necessitate that a forensic laboratory perform developmental and internal validations for methods and procedures used in forensic analysis (14).

The aim of this study is to validate the BodePlex 3 STR multiplex system on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) to be used at The Bode Technology Group (Springfield, VA) for forensic DNA analysis of small amplicons and LCN DNA. This project was designed to assess the reliability of the typing system and identify potential limitations in the analysis of smaller quantities of DNA. Additionally, it is especially valuable to familiarize users with particular limitations of a new typing system prior to taking on casework. Validation studies help a laboratory in establishing interpretation criteria for a particular typing system, such as minimum and maximum peak value thresholds, heterozygote balance ratio, allele drop out frequency and stutter peak expectations. Prior to starting the validation studies on BodePlex 3, the final stages of development were completed. After the chosen primers were tested in monoplexes, optimum primer concentrations were chosen based on the results. Primer concentrations were tested and verified in different multiplexes, and an allelic ladder was developed to size the different alleles of the STR loci. Validation experiments were performed according to the standards set forth by the DNA Advisory Board (DAB). The accuracy, precision and reproducibility of the system in typing LCN DNA were tested. DNA from different species was typed to examine the specificity of the multiplex for typing human DNA. Moreover, sensitivity experiments were conducted

in order to determine the minimum quantity of DNA that produced accurate genotyping results or interpretable partial profiles with BodePlex 3 multiplex. In addition, mixture studies were used to evaluate the reliability of the typing system in the presence of mixed DNA specimens. This project served to complete the final steps in the development of BodePlex 3 STR multiplex, and provided the necessary validation data required by DAB for forensic DNA testing laboratories.

CHAPTER II

MATERIALS AND METHODS

DNA Samples

Samples used in all the experiments include male and female DNA standards 9948 and 9947A, respectively (Coriell Cell Repositories, Camden, NJ), 39 DNA samples extracted from liquid blood, and 16 purified punches of blood samples on FTA® paper. All DNA samples used in this validation study had previously been genotyped. For the species specificity experiment, DNA samples from the following 15 different species were used: beaver, bovine, C. albicans, chicken, cottontail rabbit, coyote, dog, E. coli, mink, monkey, mouse, pig, rabbit, rat, and salmon. All the samples were provided by The Bode Technology Group (Springfield, VA).

DNA Extraction

The 16 blood samples on FTA® paper were the only sample type that needed DNA extraction. A 1.2 mm diameter portion of the bloodstain from each FTA® paper (Whatman, Clifton, NJ) was punched out into each well of a tray, and the punches were subjected to a proprietary washing process that included washes with FTA® Purification Reagent (Whatman, Clifton, NJ) and a wash buffer. The tray was then placed in an

incubator at 56°C for 1 hour to dry the punches. A reagent blank control accompanied the entire extraction procedure.

Dilutions

All the samples used had been previously quantitated at The Bode Technology Group, using PicoGreen® double-stranded DNA quantitation reagent (Molecular Probes Inc., Eugene, OR) (15) and CytoFluor® microplate reader (Applied Biosystems, Foster City, CA). DNA samples used in all the experiments were diluted in TE^{-4} buffer (10mM Tris-HCl, pH 7.5 and 0.1 mM EDTA, pH 8.0) to a 0.25 ng/µl final concentration, except in the sensitivity and species specificity experiments. For the sensitivity experiment, samples were diluted in TE^{-4} buffer into decreasing concentrations, starting at 2 ng/µl and down to 1 ng/µl, 0.5 ng/µl, 0.25 ng/µl, 0.1 ng/µl, 0.05 ng/µl, 0.025 ng/µl, 0.01 ng/µl, and 0.005 ng/µl. The different species samples were tested at 0.25 ng/µl and 5 ng/µl DNA concentrations.

Polymerase Chain Reaction (PCR)

For the primer evaluation and ladder development experiments, single loci were amplified in PCR monoplexes. In the multiplex testing experiments and the validation studies, all six microsatellites of BodePlex 3 system were amplified in one PCR multiplex reaction. The loci were: D3S1358, TH01, D5S818, D13S317, vWA and D8S1179 (Table 1). The DNA samples were amplified on a Perkin Elmer GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA). The total reaction volume was 25 µl in all

experiments. Each reaction mix contained sterilized dH_2O, amplification buffer (10 ml stock buffer containing: 7.3 ml sterilized dH_2O, 2.5 ml 10X PCR buffer [Applied Biosystems, Foster City, CA] containing 15 mM $MgCl_2$, and 50 µl 100 mM of each dNTP), 5 units AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA) and the specific primers for each locus. Primer sequences and concentrations are proprietary of The Bode Technology Group (Springfield, VA). All forward primers were labeled at the 5' end with one of three fluorescent tags: Fluorescein (blue fluorescence), HEX™ (green fluorescence), and NED™ (yellow fluorescence). The amount of DNA in each reaction was 0.25 ng unless otherwise stated. Positive (9948 and 9947A control DNA) and negative controls (sterilized dH_2O) accompanied each PCR amplification procedure. In addition, a reagent blank control for the FTA® extraction was also amplified with the FTA® samples. Thirty cycles of amplification were used in all PCR experiments, except when amplifying the FTA® samples; in this case only 23 cycles were used. The cycling parameters are proprietary of The Bode Technology Group (Springfield, VA).

Capillary Electrophoresis (CE)

PCR amplification products were subjected to capillary electrophoresis on the ABI PRISM® 3100 Genetic Analyzer (ABI 3100) (Applied Biosystems, Foster City, CA). A mixture of 10 µl Hi-Di™ formamide (Applied Biosystems, Foster City, CA) and 0.1 µl GeneScan™ –500 ROX™ Size Standard (Applied Biosystems, Foster City, CA) was prepared for each reaction. Seven-tenths of 1 µl of the PCR amplification product was

added to 10 µl of the formamide mixture in a MicroAmp® Optical 96-well reaction plate (Applied Biosystems, Foster City, CA) followed by brief centrifugation. The samples were then denatured for 5 minutes at 95 °C and subsequently snap-cooled for 2 minutes on ice. An allelic ladder was developed to size the different alleles of the STR loci, and 1 µl of the ladder was added at least once to each tray. The sample tray was loaded and the ABI 3100 was set to run. The run was conducted using dye set F, the run module "GeneScan36_POP4 Default Module", and the analysis module "GS500 Analysis".

Analysis

The data collected from the CE instrument was analyzed with GeneScan® Analysis software (16) and Genotyper® Software v 3.7 (17) (Applied Biosystems, Foster City, CA). The Genotyper® Macro used in the analysis is specific to the use of BodePlex 3, was developed by The Bode Technology Group (Springfield, VA), and is proprietary. GeneScan™ –500 ROX™ Size Standard served as the internal lane standard for sizing the different DNA fragments. The relative fluorescence unit (RFU) threshold was set at 75 during this project. Genotypes, allele base pair sizes, and peak heights (measured in RFU values) were collected and reported for all samples.

Statistical Analysis

For the results of the accuracy experiments, averages of the total alleles called by BodePlex 3 and the Profiler Plus®/COfiler® systems were calculated. Mean, standard deviation, minimum, maximum, and the range for base pair sizes of all alleles were

calculated for the results of the precision experiments. After analyzing the sensitivity experiment results, average values for peak heights (in RFU values) were calculated for all the alleles detected. Percent of heterozygote peak balance was calculated for all heterozygous loci, as: %Balance = $[(\text{peak height})_{min}/(\text{peak height})_{max}] \times 100$, where $(\text{peak height})_{min}$ and $(\text{peak height})_{max}$ are the peak height RFU values of the smaller and larger peaks, respectively. A value of 0 indicates allelic drop out and a value of 100 indicates perfectly balanced peaks at the heterozygote locus. In addition, percent of dye pull-up was calculated in all cases were pull-up occurred from one color to another, and the total average percent was reported for each dye color.

CHAPTER III

INTERNSHIP PRACTICUM JOURNAL

May 1, 2003

- Meeting with Dr. James Schumm and Robyn Wingrove at The Bode Technology Group (Springfield, VA). Discussed the internship project and decided on the validation of BodePlex 3 STR multiplex system.
- The validation study will follow the guidelines set by the DNA Advisory Board (DAB).
- Steps involved in the validation:
    1. Developmental validation:
        - Accuracy, precision and reproducibility of the system.
        - Species specificity.
        - Mixture studies.
        - Sensitivity.
        - Stability.
    2. Internal validation.

May 2, 2003

- Started training under the supervision of Robyn Wingrove.

- Learned the protocols followed by the laboratory:
    1. Polymerase chain reaction (PCR) setup.
    2. Sample preparation for separation and detection on the ABI PRISM® 3100 Genetic Analyzer (ABI 3100) (Applied Biosystems, Foster City, CA).
    3. Operating the ABI 3100.
    4. Analysis with GeneScan® Analysis and Genotyper® v 3.7 softwares (Applied Biosystems, Foster City, CA).

May 5, 2003

- Performed DNA typing with BodePlex 1 and 2 PCR amplification systems, for samples extracted from fingerprints.

May 6, 2003

- Learned DNA extraction using DNA IQ™ System (Promega Corporation, Madison, WI) on the Biomek® 2000 Laboratory Automation Workstation (Beckman Coulter, Inc.).
- Learned DNA extraction from liquid blood using QIAamp DNA Blood Midi Kit (QIAGEN Inc., Valencia, CA).

May 7, 2003

- Extracted DNA from separated epithelial and sperm cell fractions using DNA IQ™ System on the Biomek® 2000 Laboratory Automation Workstation.
- Quantitation of the extracted DNA using PicoGreen® double-stranded DNA quantitation reagent (Molecular Probes Inc., Eugene, OR) and CytoFluor® microplate reader (Applied Biosystems, Foster City, CA).

May 8, 2003

- Amplification of the DNA extracts (from May 7, 2003) using COfiler® PCR amplification kit (Applied Biosystems, Foster City, CA).
- The fragments of the amplification products were separated and detected on the ABI 3100.

May 14, 2003

- Repeated the quantitation of extracted DNA using PicoGreen® quantitation reagent and CytoFluor® microplate reader.

May 15, 2003

- Analysis with GeneScan® Analysis and Genotyper® v 3.7 softwares.

May 16, 2003

- Performed DNA typing with BodePlex 1 and 2 PCR amplification systems, for another set of samples extracted from fingerprints.
- The size fragments of the DNA samples were separated and detected on the ABI 3100.

May 19, 2003

- Analysis of the fingerprint experiment results from May 16, 2003.
- End of training period.

May 23, 2003

- Tested a series of vWA –OH primers with the fluorescent primer for the BodePlex 3 multiplex system.
- Amplified 0.25 ng 9947A and 9948 DNA templates separately with each vWA primer pair in duplicate.

May 28, 2003

- The size fragments of the amplification products from the vWA primer testing experiment were separated and detected on ABI 3100.

May 29, 2003

- Analysis of the results of vWA primer testing experiment.

- Decided on the best vWA primer pair to be included in BodePlex 3 multiplex amplification system.
- The primer pair that was decided upon yielded the highest peak heights, with the best peak morphology and the least amount of artifacts. The other primer pairs resulted in split peaks, shoulder peaks, dye pull-up, and n-1 artifacts.

May 30, 2003

- Tested varying concentrations of the selected vWA primer pair in multiplex with the other primer pairs for the remaining five loci of the BodePlex 3 system, to determine the optimum concentration.
- Prepared DNA dilutions of the 39 samples extracted from liquid blood, for use in the accuracy experiment of the validation study.
- DNA was diluted in TE^{-4} buffer to a 0.25 ng/μl final concentration.

June 2, 2003

- The size fragments of the DNA samples from the multiplex experiment were separated and detected on ABI 3100.
- Prepared allelic ladder for use with the BodePlex 3 multiplex.
- The ladder development procedure is proprietary of The Bode Technology Group (Springfield, VA).

June 3, 2003

- Analysis of the results of the multiplex experiment.
- Decided upon the optimum concentration of vWA primer pair to be used with BodePlex 3 multiplex.

June 4, 2003

- The size fragments of the ladder were separated and detected on ABI 3100.

June 5, 2003

- Analysis of the results of the ladder experiment.

June 18, 2003

- The results were successful, and the alleles for all six loci of the BodePlex 3 multiplex were well separated.
- The BodePlex 3 multiplex was ready for validation.

June 19, 2003

- Extraction of the 16 FTA® punches for use in the accuracy experiment of validation.
- Prepared new dilutions of the 39 DNA samples extracted from liquid blood (samples were diluted in TE^{-4} buffer to a final concentration of 0.25 ng/μl)

- Mixture samples were prepared in the following 9947A to 9948 template DNA ratios: 100:0, 95:5, 90:10, 80:20, 50:50, 20:80, 10:90, 5:95, and 0:100.

June 20, 2003

- All validation study samples were amplified with BodePlex 3 multiplex.
- A DNA template of 0.25 ng/μl was used in all amplifications; except in the sensitivity and species specificity experiments.
- For the sensitivity study, the following template amounts were amplified with the multiplex: 2 ng/μl, 1 ng/μl, 0.5 ng/μl, 0.25 ng/μl, 0.1 ng/μl, 0.05 ng/μl, 0.025 ng/μl, 0.01 ng/μl, and 0.005 ng/μl.
- The different species samples were tested at 0.25 ng/μl and 5 ng/μl DNA concentrations.
- Negative and positive controls accompanied all amplifications.

June 23, 2003

- DNA fragments of amplification products from the following experiments were separated and detected on the ABI 3100: accuracy, sensitivity, and mixture studies.

June 24, 2003

- DNA fragments of amplification products from the following experiments were separated and detected on the ABI 3100: reproducibility and species specificity studies.

June 25, 2003

- Analysis of results from all validation experiments.
- Re-analysis of some samples on the ABI 3100.

June 26, 2003

- Analysis of the results from repeated experiments.
- Validation study completed.

CHAPTER IV

RESULTS

Final stages of BodePlex 3 multiplex development

Prior to starting the validation studies on BodePlex 3 system, the final stages of multiplex development were carried out. All primer sequences and concentrations used in these experiments, as well as the development of the allelic ladder, are proprietary of The Bode Technology Group (Springfield, VA). A series of different vWA –OH primers was tested in monoplex with the chosen fluorescent primer (18). The testing was carried out by amplifying 0.25 ng of 9947A and 9948 DNA templates with each vWA primer pair. All amplifications were carried out in duplicate, and negative controls accompanied all the procedures. The amplification products were analyzed on the ABI PRISM® 3100 Genetic Analyzer (ABI 3 100) (Applied Biosystems, Foster City, CA). The primer pair that was decided upon yielded the highest peak heights, with the best peak morphology and the least amount of artifacts. The other primer pairs resulted in split peaks, shoulder peaks, dye pull-up, and non-template nucleotide addition. The primer pair was then tested in multiplex with the other primers for the remaining five loci of the BodePlex 3 system. The next step was to develop an allelic ladder to size the different alleles generated by the multiplex; this procedure is also proprietary of The Bode Technology Group. Ladder development experiments were carried out and an allelic ladder was successfully

developed (Figure 1), as well as a Genotyper® Macro to analyze the data. Following multiplex optimization experiments, the BodePlex 3 system was ready for validation (Figure 2).

Accuracy

The 16 purified FTA® punches and the 39 DNA samples, extracted from liquid blood, were amplified with BodePlex 3 multiplex. Allele calls were determined for all the samples, and compared to previously determined genotypes by AmpF*l*STR® Profiler Plus® and COfiler® (Applied Biosystems, Foster City, CA) PCR amplification kits. For all 16 FTA samples all the allele calls were identical between the systems. In addition, with BodePlex 3 two additional alleles were called for one of the samples (sample 6571) at the TH01 locus, which previously did not give a genotype with the COfiler® system (Table 2). In the case of the 39 liquid blood samples, three samples (7290, 7303, and 7317) gave genotypes at the TH01 locus that could not be previously detected with the COfiler® system (Table 3). Allelic drop out occurred at the TH01 locus in sample 7304 with the BodePlex 3 system, and in sample 7311 with the COfiler® system. Moreover, samples 7310 and 7322 gave discordant genotypes between BodePlex 3 and COfiler® systems, both at the TH01 locus. Sample 7310 gave a (9.3, 9.3) genotype with BodePlex 3 and a (6, 9) with the COfiler® system, and sample 7322 gave a (7, 9.3) genotype with BodePlex 3 and a (6, 9.3) with the COfiler® system. However, when typed with the PowerPlex® 16 system (Promega Corporation, Madison, WI), the genotypes were identical between BodePlex 3 and PowerPlex® 16 systems. Overall more alleles were

called with the BodePlex 3 multiplex despite the use of 0.25 ng template DNA versus 1 ng template for the Profiler Plus®/COfiler® systems.

Precision

To evaluate the precision of BodePlex 3 typing system, sixteen samples of 0.25 ng human control DNA 9947A were amplified separately with the multiplex. The amplified fragments of each sample were injected twice each and separated on the ABI 3100. In addition, sixteen allelic ladders were injected twice each and separated on the ABI 3100. The base pair size for each allele was determined for all the 9947A samples and all the ladders using Genotyper ® Software v 3.7. The standard deviation of base pair sizes for all the loci was between 0.03 and 0.06 bp for the sixteen 9947A samples (Table 4), and between 0.03 and 0.17 bp for the sixteen ladders (Table 5). These values represent both injections performed.

Reproducibility

Sixteen samples of 0.25 ng human control DNA 9947A were amplified separately with BodePlex 3 multiplex. The amplified fragments of each 9947A sample were then injected twice each and separated on the ABI 3100. Allele calls for all the amplified and detected 9947A samples were identical regardless of the ABI 3100 capillary position or injection (Table 6).

Species specificity

Samples from 15 different species were amplified with BodePlex 3 multiplex using DNA templates at two different concentrations, 0.25 ng and 5 ng (Table 7). The amplification products were then analyzed on the ABI 3100 to determine if any of the different species DNA yielded allele peaks when amplified with BodePlex 3 primer system. The chicken (0.25 ng), E. coli (5 ng), monkey (5 ng), mouse (5 ng), and rabbit (5 ng) DNA generated peaks in regions of human alleles, with peak heights of 56, 59, 181, 104, and 217 relative fluorescence units (RFU), respectively. All the other species tested did not produce peaks in these regions (Table 8). Moreover, it cannot be excluded that other untested species DNA may generate different peaks with the use of the BodePlex 3 primers.

Sensitivity

The following nine different concentrations of 9947A and 9948 DNA templates were amplified with the BodePlex 3 multiplex: 2 ng, 1 ng, 0.5 ng, 0.25 ng, 0.1 ng, 0.05 ng, 0.025 ng, 0.01 ng, and 0.005 ng. All amplifications were carried out in triplicate. Three samples with 0.005 ng and two samples with 0.01 ng template amounts generated peaks with average heights above 100 RFU at at least one locus (Table 9). Complete profiles were observed for two 9948 samples with as little as 0.025ng DNA template, and for one 9948 sample with 0.05 ng template. However it is not recommended to use this amount when full balanced profiles are required. At 0.1 ng of DNA template allelic drop out at the TH01 locus occurred in three samples (Table 10). DNA amounts of 0.25 ng and 0.5 ng produced balanced profiles for all the samples analyzed; the total average peak heights

for all the loci ranged from 1142 to 4128 RFU, and no measurable dye pull-up (exceeding 50 RFU) was observed (Figure 3). With 1 ng and 2 ng DNA templates signal saturation occurred, with substantial dye pull-up (Table 11), and high levels of stutter. Correct allele calls were made for all 9947A samples over the range of 0.25 ng to 2 ng, and over the range of 0.1 ng to 1 ng for all 9948 samples. As expected, average peak height values increased with increasing DNA template amounts (Figure 4). Moreover, for all samples analyzed, allelic drop out began to be observed at 0.1 ng template amounts and continued down to 0.005 ng (primarily at the TH01 locus). In addition, average peak height balance at heterozygote loci decreased with lower template amounts (Figure 5 and Table 12).

Mixtures

Nine DNA mixtures of 0.25 ng total template amount were prepared from 9947A and 9948 human control DNA. The two different DNA samples share alleles at all six STR loci of BodePlex 3 multiplex, and have the same genotype at one locus (Table 13). The mixtures were prepared in the following 9947A to 9948 ratios: 100:0, 95:5, 90:10, 80:20, 50:50, 20:80, 10:90, 5:95, and 0:100. The mixture samples were then amplified with BodePlex 3 multiplex and analyzed on the ABI 3100. Peak heights of the different alleles observed in each mixture sample were determined in RFU values (Table 14). The resulting genotypes were evaluated for the presence of the following mixture indicators: more than two peaks at a locus, peaks at stutter positions (4 base pairs below the main peak) with higher RFU values than typical stutter peaks ($\leq$ 17.5% of the larger peak), and heterozygote peak imbalance. All mixture samples (except the 100:0 and the 0:100

samples) displayed three peaks at the D3S1358 locus (both samples have a heterozygote profile at this locus) with RFU values above 100 RFU, except for a 62 RFU peak for the minor component in the 95:5 mixture sample. Only the 90:10 and the 50:50 samples displayed three peaks at the TH01 locus, although it was expected to obtain three peaks in other mixture samples as well, but this was not the case due to the high level of allelic drop out at this locus. The (12, 13) genotype at the D8S1179 locus of the minor component profile in the 95:5 mixture could not be called because the 12 allele was at a stutter position with a peak height value characteristic of a stutter peak. Peak height proportions were all indicative of the presence of a mixture at all the loci (except TH01) in the following mixture samples: 80:20, 50:50, and 20:80. The other samples also displayed significant peak height imbalance (indicative of the presence of a mixed sample), but not at all the loci. It was possible to predict the minor component's partial profile in the 95:5, 5:95, 10:90, 80:20 and 20:80 mixtures, and full profiles of both components in the 90:10 and 50:50 mixture samples (Figure 6).

Throughout all the development experiments and validation studies, all the negative and positive controls produced the expected results, and no sporadic allele drop in events were observed in any of the experiments.

CHAPTER V

DISCUSSION

With the advancement of forensic DNA typing over the years, research in the area of human DNA identification continues to progress toward the development of more robust, efficient and sensitive techniques. Forensic DNA profiling is now largely established in courts as important evidence, and databases of DNA profiles are being implemented in many countries (4). The challenges faced with the quality of biological samples being analyzed necessitate the improvement of current methods and protocols. The most common of these challenges is the analysis of compromised biological samples. When the DNA in a specimen is extremely degraded or present in low quantities, the sensitivity of a typing system becomes a critical issue.

The BodePlex 3 mini-STR typing system was developed for use with compromised samples of degraded and low copy number (LCN) DNA. The multiplex primers were specifically designed to target smaller fragments of template DNA by bringing the complementary primer strands closer to the target region and hence decreasing the amplicon size. This approach increases the chances of detecting genetic profiles when the DNA template is degraded or present in small quantities.

The standards set forth by the DNA Advisory Board (DAB) require that a forensic DNA testing laboratory perform validation studies on novel techniques or procedures prior to implementation for casework analysis. Validation studies help familiarize users with the various potentials and limitations of a forensic DNA typing system. These studies also help to set the grounds for establishing interpretation criteria such as minimum and maximum peak value thresholds, heterozygote balance ratio, and allelic drop out frequency. The purpose of this study was to validate the BodePlex 3 mini-STR multiplex system on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) to be used at The Bode Technology Group (Springfield, VA) for forensic DNA analysis of small amplicons and LCN DNA. The project and experiments performed were designed according to DAB recommendations and standards.

Preceding the validation studies, the final stages of BodePlex 3 multiplex development were completed. The validation studies were conducted and focused on evaluating the accuracy, precision, reproducibility, species specificity, sensitivity and mixture resolving capacity of the typing system. The accuracy experiments performed demonstrate the system's ability at accurately detecting all the alleles of the tested samples. Except for one allelic drop out event at the TH01 locus (Table 3), all alleles were typed correctly. Moreover, discrepancies for two samples were observed between the BodePlex 3 system and the Profiler Plus®/COfiler® systems, also at the TH01 locus (Table 3). However, when typed with the PowerPlex® 16 system, both samples gave identical allele calls between BodePlex 3 and PowerPlex® 16 systems. These discrepancies can not be explained at this point, and additional testing is currently

underway at The Bode Technology Group. In addition, more total alleles were called with the BodePlex 3 system versus the Profiler Plus®/COfiler® systems, despite the use of 0.25 ng template DNA as opposed to 1 ng template for the Profiler Plus®/COfiler® systems. This shows that the BodePlex 3 system is more accurate and sensitive than the Profiler Plus®/COfiler® systems, even at lower template amounts.

The precision and reproducibility of the typing system were tested and assessed. Standard deviation of base pair sizes determined for all the loci was between 0.03 and 0.06 bp for the sixteen 9947A samples tested, and between 0.03 and 0.17 bp for the sixteen allelic ladders; for the two separate injections performed (Tables 4 and 5). These values indicate the precision of the system in determining allelic base pair sizes. In addition, allele calls for 9947A DNA template were reproducible 32 separate times; 16 times in each injection performed (Table 6).

Decreasing amounts of 9947A and 9948 DNA templates were amplified with the multiplex to evaluate the sensitivity of BodePlex 3. The 0.25 ng and 0.5 ng samples produced the most balanced profiles for both 9947A and 9948 DNA (Figure 3); the profiles were reproduced in three separate amplifications for each template amount. Although complete profiles were observed for two of the three replicates with as little as 0.025ng DNA template, and for one sample with 0.05 ng template, these amounts are not recommended for use when full balanced profiles are required. Due to the high sensitivity of the system, templates of 1 ng and 2 ng DNA resulted in signal saturation and high incidences of dye pull-up (primarily from the yellow pulling up into the green color) (Table 11), and stutter. In general, stutter occurrences were minimal with BodePlex 3

system. In addition, for all samples analyzed, allelic drop out started at 0.1 ng template amounts and down to 0.005 ng (primarily at the TH01 locus), and as expected, average peak height balance at heterozygote loci decreased with lower template amounts (Figure 5 and Table 12). While the smaller allele at a heterozygote locus is expected to be at least 30% of the larger one at peak heights exceeding 600 RFU, even more pronounced heterozygote allele imbalance is not unexpected with peak heights of lower RFU values. This can be attributed to the fact that with low template amounts, one allele of a heterozygous locus can be preferentially amplified due to chance, and can result in a higher signal intensity or peak height. Moreover, average peak height values (in RFU) increased with increasing DNA template amounts (Figure 4). Based on these results, the following conclusions and recommendations can be made: homozygotes may be called if an individual peak at a locus exceeds 200 RFU; allele drop out was not observed with homozygotes above 132 RFU. Heterozygote alleles may be called as long as they exceed 50 RFU. If a single allele peak height value falls below the homozygote cut off, it can be called as part of a partial profile. Although a full profile might not be generated, this will help when matching profiles.

Allele drop in events were not observed in any of the negative controls (sterilized dH_2O templates, under the same amplification conditions). However, since it is an unpredictable event, to avoid potential error from sporadic allele drop in events, the amplification may be performed in duplicate, since the chances of allele drop in events being precisely replicated in two separate amplifications are exceedingly small.

In the species specificity experiments, five different non-human DNA samples (chicken, E. coli, monkey, mouse, and rabbit) generated single peaks in regions of human alleles, with peak heights ranging from 56 to 217 relative fluorescence units (RFU) (Table 8). All the other species tested did not produce peaks in these regions. The allelic peak heights generated by amplification of 0.25 ng chicken DNA and 5 ng of each of E. coli, monkey, and mouse DNA, were below 200 RFU; hence, in casework scenario, these peaks will not be called. The allele peak generated in an area of human alleles by the 5 ng rabbit sample had a peak height value above 200 RFU. However, unless a biological specimen submitted for human identification is contaminated with 5 ng or more of monkey, mouse, or rabbit DNA, these peaks are not expected to be reproduced. Moreover, it cannot be excluded that other untested species DNA may generate different peaks with the use of the BodePlex 3 primers. Inspite of these results, the BodePlex 3 system remains highly specific for human DNA typing.

To determine the ability of the BodePlex 3 typing system at resolving mixed DNA profiles, different mixture samples of 9947A and 9948 DNA templates were prepared and amplified with the multiplex. It was possible to predict the minor component's partial profile in the 95:5, 5:95, 10:90, 80:20 and 20:80 mixtures, and full profiles of both components in the 90:10 and 50:50 mixture samples (Figure 6). Even at the low template amounts used in this experiment (0.25 ng DNA), the BodePlex 3 system was able to detect the presence of a mixed DNA sample. Moreover, it is not unusual for allelic drop out events to occur at these low levels of DNA template; for example, in the

95:5 mixture sample, the DNA amount of the minor component is 5% of 0.25 ng, or 0.0125 ng.

When interpreting DNA profiles generated by the BodePlex 3 mini-STR typing system, it is recommended to be conservative. Homozygote allele peaks can be called at or above 200 RFU, and heterozygotes at or above 50 RFU, with confidence. Allelic drop out is very frequent with degraded or LCN DNA templates, even for higher peak heights. Variations in the primer-binding sequence of a DNA template can result in failure of primer hybridization, amplification and subsequent detection of an existing allele (1). In addition, one allele of a heterozygote locus may be amplified by chance during the early rounds of PCR and is more likely to be preferentially amplified and detected, resulting in drop out of the other allele. Moreover, when dealing with compromised biological specimens, the total amount of intact DNA present must be taken into account. This factor can have stochastic effects, which can adversely affect the resulting DNA profile. In addition, laboratory-based contamination is not unexpected, due to the high sensitivity of the system in detecting low DNA levels. As with all typing systems, special care must be taken to avoid contamination. Other aspects to be considered are allele peak height balance at heterozygote loci, occurrence of dye blobs, and stutter incidences.

This study served to validate the use of BodePlex 3 mini-STR multiplex system on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) at The Bode Technology Group (Springfield, VA) in forensic DNA analysis of small amplicons and LCN DNA. It fulfilled the primary requirements set forth by the DNA Advisory Board (DAB) for forensic DNA testing laboratories and defined possible

limitations that must be considered for successful use and interpretation of DNA profiling results. Performance of the multiplex was accurate, reliable and reproducible. The results indicated that the BodePlex 3 system is highly specific for typing human DNA, sensitive for detecting DNA profiles of LCN DNA, and is capable of resolving mixtures to a certain extent. In conclusion, BodePlex 3 mini-STR typing system is robust for the analysis of LCN human DNA and is ready for use in forensic casework analysis.

CHAPTER VI

TABLES AND FIGURES

Table 1. Summary of BodePlex 3 mini-STR multiplex loci. (a) Alleles and base pair sizes are those observed in the current study. Table (b) represents a comparison of allele base pair sizes between BodePlex 3, Profiler Plus™, COfiler®, and PowerPlex® 16 systems.

a. BodePlex 3 mini-STR multiplex loci.

Locus	Dye	Color	Alleles	Base pair sizes
D3S1358	Fluorescein	Blue	12-19	112-141
TH01	Fluorescein	Blue	4-9, 9.3, 10, 11, 13.3	155-194
D5S818	HEX™	Green	7-16	114-152
D13S317	HEX™	Green	8-15	178-206
vWA	NED™	Yellow	11-24	137-191
D8S1179	NED™	Yellow	7-18	202-246

b. Comparison of base pair sizes.

System	BodePlex 3	Profiler Plus™	COfiler®	PowerPlex® 16
D3S1358	112-141	114-142	114-142	115-147
TH01	155-194		169-189	156-195
D5S818	114-152	135-171		119-155
D13S317	178-206	206-234		176-208
vWA	137-191	157-197		123-171
D8S1179	202-246	128-168		203-247

Table 2. BodePlex 3 accuracy - FTA® samples. Genotype comparison of the 16 FTA® samples amplified with BodePlex 3 (BP3) and Profiler Plus®/COfiler® systems (Pro/CO). Highlighted cells denote allelic drop out.

System	Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2	BP3 Alleles called	Pro/CO Alleles called	Excess BP3 alleles	Excess Pro/CO alleles
BP3	6535	15	16	7	9.3	11	12	11	12	17	18	13	16	12			
Pro/CO	6535	15	16	7	9.3	11	12	11	12	17	18	13	16		12		
BP3	6546	14	15	7	9.3	11	12	9	11	17	20	12	13	12			
Pro/CO	6546	14	15	7	9.3	11	12	9	11	17	20	12	13		12		
BP3	6547	16	17	9.3	9.3	10	12	11	13	17	18	11	12	12			
Pro/CO	6547	16	17	9.3	9.3	10	12	11	13	17	18	11	12		12		
BP3	6549	16	17	6	7	12	12	9	9	18	19	8	11	12			
Pro/CO	6549	16	17	6	7	12	12	9	9	18	19	8	11		12		
BP3	6551	16	16	9	9	11	13	9	12	15	18	13	14	12			
Pro/CO	6551	16	16	9	9	11	13	9	12	15	18	13	14		12		
BP3	6571	16	16	6	9.3	13	13	8	13	18	19	12	13	12		2	
Pro/CO	6571	16	16	0	0	13	13	8	13	18	19	12	13		10		
BP3	6579	14	16	9.3	10	11	12	11	12	17	17	12	13	12			
Pro/CO	6579	14	16	9.3	10	11	12	11	12	17	17	12	13		12		
BP3	6580	14	16	7	8	11	13	11	11	14	17	10	13	12			
Pro/CO	6580	14	16	7	8	11	13	11	11	14	17	10	13		12		
BP3	6581	16	16	9.3	9.3	11	11	11	13	16	17	11	15	12			
Pro/CO	6581	16	16	9.3	9.3	11	11	11	13	16	17	11	15		12		
BP3	6582	14	17	7	9.3	9	10	9	11	17	18	13	15	12			
Pro/CO	6582	14	17	7	9.3	9	10	9	11	17	18	13	15		12		
BP3	6621	16	18	9	9.3	12	12	8	13	17	18	10	14	12			
Pro/CO	6621	16	18	9	9.3	12	12	8	13	17	18	10	14		12		
BP3	6629	14	18	6	9.3	11	12	10	11	15	15	14	14	12			
Pro/CO	6629	14	18	6	9.3	11	12	10	11	15	15	14	14		12		
BP3	6638	17	18	6	9.3	11	12	10	13	15	16	13	15	12			
Pro/CO	6638	17	18	6	9.3	11	12	10	13	15	16	13	15		12		
BP3	6644	14	16	7	9.3	9	13	8	10	19	19	12	14	12			
Pro/CO	6644	14	16	7	9.3	9	13	8	10	19	19	12	14		12		
BP3	6648	17	18	6	6	12	13	12	12	14	16	10	12	12			
Pro/CO	6648	17	18	6	6	12	13	12	12	14	16	10	12		12		
BP3	6667	16	17	9	9.3	12	13	9	11	16	18	14	16	12			
Pro/CO	6667	16	17	9	9.3	12	13	9	11	16	18	14	16		12		
													Total alleles	192	190	2	0
													Average	12	11.88	0.13	0

Table 3. BodePlex 3 accuracy - DNA samples from liquid blood. Genotype comparison of the 39 DNA samples extracted from liquid blood, amplified with BodePlex 3 (BP3) and Profiler Plus®/COfiler® systems (Pro/CO). Highlighted cells denote allelic drop out. Cells shaded in green indicate a discrepancy between the systems.

System	Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2	BP3 Alleles called	Pro/CO Alleles called	Excess BP3 alleles	Excess Pro/CO alleles
BP3	7283	16	18	6	7	11	12	12	12	16	17	13	13	12			
Pro/CO	7283	16	18	6	7	11	12	12	12	16	17	13	13		12		
BP3	7284	16	16	7	9.3	12	13	11	12	16	16	14	14	12			
Pro/CO	7284	16	16	7	9.3	12	13	11	12	16	16	14	14		12		
BP3	7285	14	17	6	9	11	12	12	13	16	17	13	13	12			
Pro/CO	7285	14	17	6	9	11	12	12	13	16	17	13	13		12		
BP3	7286	16	17	9	9	12	13	8	11	14	17	10	16	12			
Pro/CO	7286	16	17	9	9	12	13	8	11	14	17	10	16		12		
BP3	7287	16	17	7	9	7	9	12	14	15	16	14	14	12			
Pro/CO	7287	16	17	7	9	7	9	12	14	15	16	14	14		12		
BP3	7288	14	14	6	6	11	12	11	12	16	17	14	14	12			
Pro/CO	7288	14	14	6	6	11	12	11	12	16	17	14	14		12		
BP3	7289	15	17	9	9.3	11	13	11	12	16	19	12	13	12			
Pro/CO	7289	15	17	9	9.3	11	13	11	12	16	19	12	13		12		
BP3	7290	15	18	9.3	9.3	11	13	10	11	14	17	13	14	12		2	
Pro/CO	7290	15	18	0	0	11	13	10	11	14	17	13	14		10		
BP3	7291	16	17	6	7	11	12	11	13	16	17	12	13	12			
Pro/CO	7291	16	17	6	7	11	12	11	13	16	17	12	13		12		
BP3	7292	14	16	6	7	12	12	11	12	15	15	13	14	12			
Pro/CO	7292	14	16	6	7	12	12	11	12	15	15	13	14		12		
BP3	7293	17	18	6	9.3	11	12	10	10	15	18	11	14	12			
Pro/CO	7293	17	18	6	9.3	11	12	10	10	15	18	11	14		12		
BP3	7294	15	16	6	6	9	12	8	12	15	15	12	14	12			
Pro/CO	7294	15	16	6	6	9	12	8	12	15	15	12	14		12		
BP3	7295	15	15	6	7	11	12	11	12	17	19	11	13	12			
Pro/CO	7295	15	15	6	7	11	12	11	12	17	19	11	13		12		
BP3	7296	17	17	9.3	9.3	11	12	11	11	16	18	14	15	12			
Pro/CO	7296	17	17	9.3	9.3	11	12	11	11	16	18	14	15		12		
BP3	7297	17	17	6	8	11	12	11	13	17	17	12	15	12			
Pro/CO	7297	17	17	6	8	11	12	11	13	17	17	12	15		12		
BP3	7298	17	17	7	8	10	13	11	12	16	18	13	15	12			
Pro/CO	7298	17	17	7	8	10	13	11	12	16	18	13	15		12		

Table 3. Continued.

System	Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2	BP3 Alleles called	Pro/CO Alleles called	Excess BP3 alleles	Excess Pro/CO alleles
BP3	7299	15	16	7	9.3	10	11	9	9	15	17	13	13	12			
Pro/CO	7299	15	16	7	9.3	10	11	9	9	15	17	13	13		12		
BP3	7300	15	19	7	7	11	13	8	10	17	19	11	13	12			
Pro/CO	7300	15	19	7	7	11	13	8	10	17	19	11	13		12		
BP3	7301	16	16	7	9	11	12	11	12	13	16	15	15	12			
Pro/CO	7301	16	16	7	9	11	12	11	12	13	16	15	15		12		
BP3	7302	17	17	8	9	10	12	10	12	13	17	12	14	12			
Pro/CO	7302	17	17	8	9	10	12	10	12	13	17	12	14		12		
BP3	7303	15	17	7	9	10	11	11	12	15	17	13	15	12		2	
Pro/CO	7303	15	17	0	0	10	11	11	12	15	17	13	15		10		
BP3	7304	16	16	7	7	11	13	10	12	18	18	14	15	12			
Pro/CO	7304	16	16	7	9	11	13	10	12	18	18	14	15		12		
BP3	7305	16	17	6	6	11	12	10	11	16	16	12	14	12			
Pro/CO	7305	16	17	6	6	11	12	10	11	16	16	12	14		12		
BP3	7306	15	15	9	9.3	10	11	10	12	17	17	13	15	12			
Pro/CO	7306	15	15	9	9.3	10	11	10	12	17	17	13	15		12		
BP3	7307	16	18	6	9	11	12	8	12	17	17	13	14	12			
Pro/CO	7307	16	18	6	9	11	12	8	12	17	17	13	14		12		
BP3	7308	15	17	9	9.3	11	12	11	13	15	16	13	13	12			
Pro/CO	7308	15	17	9	9.3	11	12	11	13	15	16	13	13		12		
BP3	7309	16	16	9.3	9.3	11	13	14	14	14	16	13	14	12			
Pro/CO	7309	16	16	9.3	9.3	11	13	14	14	14	16	13	14		12		
BP3	7310	15	17	9.3	9.3	11	12	11	12	16	17	12	13	12			
Pro/CO	7310	15	17	6	9	11	12	11	12	16	17	12	13		12		
BP3	7311	15	18	6	9.3	11	12	9	12	15	18	12	15	12			
Pro/CO	7311	15	18	9.3	9.3	11	12	9	12	15	18	12	15		12		
BP3	7312	15	16	9.3	9.3	11	13	10	11	17	17	10	15	12			
Pro/CO	7312	15	16	9.3	9.3	11	13	10	11	17	17	10	15		12		
BP3	7313	15	15	9.3	9.3	7	12	8	11	15	16	11	13	12			
Pro/CO	7313	15	15	9.3	9.3	7	12	8	11	15	16	11	13		12		
BP3	7314	15	16	6	9.3	11	14	13	14	17	19	11	14	12			
Pro/CO	7314	15	16	6	9.3	11	14	13	14	17	19	11	14		12		
BP3	7315	16	18	6	7	11	12	11	11	14	18	13	13	12			
Pro/CO	7315	16	18	6	7	11	12	11	11	14	18	13	13		12		
BP3	7316	14	17	6	7	11	11	11	11	17	18	10	10	12			
Pro/CO	7316	14	17	6	7	11	11	11	11	17	18	10	10		12		
BP3	7317	17	17	8	9.3	13	13	8	11	15	18	10	11	12		2	
Pro/CO	7317	17	17	0	0	13	13	8	11	15	18	10	11		10		

Table 3. Continued.

System	Sample info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2	BP3 Alleles called	Pro/CO Alleles called	Excess BP3 alleles	Excess Pro/CO alleles
BP3	7318	16	16	7	7	12	12	11	11	16	16	11	13	12			
Pro/CO	7318	16	16	7	7	12	12	11	11	16	16	11	13		12		
BP3	7319	14	16	7	10	12	12	11	12	11	16	12	15	12			
Pro/CO	7319	14	16	7	10	12	12	11	12	11	16	12	15		12		
BP3	7321	15	16	6	9.3	9	11	10	12	16	18	10	14	12			
Pro/CO	7321	15	16	6	9.3	9	11	10	12	16	18	10	14		12		
BP3	7322	15	17	7	9.3	12	12	11	12	16	18	13	14	12			
Pro/CO	7322	15	17	6	9.3	12	12	11	12	16	18	13	14		12		
												Total alleles		468	462	6	0
												Average		12	11.85	0.15	0

Table 4. BodePlex 3 precision - 9947A samples. Allele base pair sizes for the 16 9947A samples amplified with BodePlex 3. Tables "a" and "b" represent the first and second injections, respectively.

a. First injection.

Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2
9947_a1	120.08	124.12	171.56	178.47	130.41	130.41	190.35	190.35	162.67	166.73	225.45	225.45
9947_b1	119.99	124.09	171.61	178.49	130.34	130.34	190.30	190.30	162.55	166.69	225.50	225.50
9947_c1	120.04	124.05	171.51	178.46	130.35	130.35	190.36	190.36	162.55	166.67	225.57	225.57
9947_d1	120.03	124.09	171.61	178.55	130.34	130.34	190.35	190.35	162.60	166.68	225.50	225.50
9947_e1	120.14	124.18	171.49	178.49	130.41	130.41	190.35	190.35	162.58	166.65	225.52	225.52
9947_f1	120.08	124.13	171.59	178.52	130.45	130.45	190.37	190.37	162.67	166.74	225.53	225.53
9947_g1	120.09	124.20	171.55	178.46	130.41	130.41	190.30	190.30	162.58	166.64	225.56	225.56
9947_h1	120.15	124.19	171.61	178.55	130.48	130.48	190.43	190.43	162.67	166.75	225.63	225.63
9947_I1	120.04	124.15	171.52	178.49	130.43	130.43	190.35	190.35	162.58	166.63	225.60	225.60
9947_j1	120.03	124.15	171.55	178.47	130.46	130.46	190.38	190.38	162.58	166.64	225.60	225.60
9947_k1	120.08	124.13	171.47	178.38	130.37	130.37	190.30	190.30	162.58	166.64	225.50	225.50
9947_L1	120.05	124.11	171.55	178.42	130.36	130.36	190.33	190.33	162.60	166.69	225.53	225.53
9947_m1	120.08	124.21	171.61	178.47	130.45	130.45	190.35	190.35	162.60	166.75	225.56	225.56
9947_n1	120.12	124.18	171.61	178.55	130.43	130.43	190.35	190.35	162.60	166.75	225.58	225.58
9947_o1	120.15	124.17	171.58	178.53	130.44	130.44	190.37	190.37	162.65	166.69	225.50	225.50
9947_p1	120.06	124.17	171.61	178.53	130.42	130.42	190.31	190.31	162.68	166.73	225.50	225.50
Mean	120.08	124.15	171.56	178.49	130.41	130.41	190.35	190.35	162.61	166.69	225.54	225.54
Std. Dev.	0.05	0.05	0.05	0.05	0.04	0.04	0.03	0.03	0.04	0.04	0.05	0.05
Min.	119.99	124.05	171.47	178.38	130.34	130.34	190.30	190.30	162.55	166.63	225.45	225.45
Max.	120.15	124.21	171.61	178.55	130.48	130.48	190.43	190.43	162.68	166.75	225.63	225.63
Range	0.16	0.16	0.14	0.17	0.14	0.14	0.13	0.13	0.13	0.12	0.18	0.18

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.03

Maximum Std. Dev. = 0.05

Table 4. Continued.

b. Second injection.

Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2
9947_a2	120.08	124.12	171.56	178.47	130.41	130.41	190.35	190.35	162.67	166.73	225.45	225.45
9947_b2	120.03	124.09	171.51	178.45	130.35	130.35	190.25	190.25	162.59	166.66	225.48	225.48
9947_c2	120.03	124.09	171.53	178.48	130.35	130.35	190.31	190.31	162.59	166.67	225.54	225.54
9947_d2	120.08	124.09	171.59	178.56	130.38	130.38	190.40	190.40	162.69	166.71	225.52	225.52
9947_e2	120.15	124.14	171.58	178.48	130.41	130.41	190.36	190.36	162.61	166.70	225.52	225.52
9947_f2	120.23	124.23	171.52	178.44	130.50	130.50	190.33	190.33	162.61	166.63	225.54	225.54
9947_g2	120.11	124.15	171.57	178.50	130.46	130.46	190.36	190.36	162.66	166.73	225.63	225.63
9947_h2	120.06	124.18	171.69	178.65	130.45	130.45	190.42	190.42	162.78	166.78	225.61	225.61
9947_I2	120.06	124.10	171.57	178.52	130.38	130.38	190.36	190.36	162.58	166.68	225.55	225.55
9947_j2	120.09	124.14	171.47	178.43	130.35	130.35	190.31	190.31	162.57	166.59	225.54	225.54
9947_k2	120.00	124.13	171.52	178.45	130.42	130.42	190.28	190.28	162.65	166.65	225.51	225.51
9947_L2	120.06	124.15	171.52	178.47	130.37	130.37	190.33	190.33	162.55	166.76	225.52	225.52
9947_m2	120.04	124.11	171.54	178.46	130.39	130.39	190.27	190.27	162.62	166.65	225.45	225.45
9947_n2	120.09	124.17	171.43	178.39	130.38	130.38	190.31	190.31	162.54	166.59	225.59	225.59
9947_o2	120.08	124.16	171.45	178.38	130.46	130.46	190.22	190.22	162.53	166.64	225.50	225.50
9947_p2	120.08	124.16	171.57	178.51	130.46	130.46	190.38	190.38	162.62	166.74	225.59	225.59
Mean	120.08	124.14	171.54	178.48	130.41	130.41	190.33	190.33	162.62	166.68	225.53	225.53
Std. Dev.	0.05	0.04	0.06	0.06	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.05
Min.	120.00	124.09	171.43	178.38	130.35	130.35	190.22	190.22	162.53	166.59	225.45	225.45
Max.	120.23	124.23	171.69	178.65	130.50	130.50	190.42	190.42	162.78	166.78	225.63	225.63
Range	0.23	0.14	0.26	0.27	0.15	0.15	0.20	0.20	0.25	0.19	0.18	0.18

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.04

Maximum Std. Dev. = 0.06

Table 5. BodePlex 3 precision - allelic ladders. Allele base pair sizes for the 16 allelic ladders. Tables "a" and "b" represent the first and second injections, respectively. Data for the alleles of each locus are presented in separate tables.

a. First injection. (D3S1358 locus)

Sample Info	D3S1358 1	D3S1358 2	D3S1358 3	D3S1358 4	D3S1358 5	D3S1358 6	D3S1358 7	D3S1358 8
Ladder_a1	111.87	116.04	119.96	123.93	128.18	132.40	136.52	140.73
Ladder_b1	112.02	116.14	120.00	124.06	128.24	132.48	136.60	140.80
Ladder_c1	112.06	116.10	120.03	124.08	128.25	132.48	136.52	140.80
Ladder_d1	111.89	115.95	119.99	123.99	128.12	132.38	136.46	140.72
Ladder_e1	111.84	115.98	119.94	123.96	128.17	132.45	136.54	140.81
Ladder_f1	112.00	116.07	120.04	124.05	128.26	132.46	136.62	140.72
Ladder_g1	111.97	116.04	120.09	124.10	128.31	132.49	136.71	140.77
Ladder_h1	112.00	116.14	120.10	124.10	128.23	132.56	136.55	140.78
Ladder_I1	111.96	116.08	120.03	124.09	128.28	132.44	136.56	140.69
Ladder_j1	112.01	116.08	120.04	124.12	128.24	132.49	136.56	140.77
Ladder_k1	111.92	116.06	120.09	124.02	128.22	132.48	136.55	140.71
Ladder_L1	111.98	116.04	120.07	124.00	128.21	132.39	136.47	140.72
Ladder_m1	112.03	116.10	120.07	124.08	128.29	132.47	136.63	140.79
Ladder_n1	112.06	116.14	120.12	124.06	128.28	132.47	136.55	140.77
Ladder_o1	111.96	116.16	120.03	124.09	128.36	132.44	136.56	140.69
Ladder_p1	111.64	115.64	119.66	123.62	127.89	132.26	136.48	140.76
Mean	111.95	116.05	120.02	124.02	128.22	132.45	136.56	140.75
Std. Dev.	0.10	0.12	0.11	0.12	0.11	0.07	0.06	0.04
Min.	111.64	115.64	119.66	123.62	127.89	132.26	136.46	140.69
Max.	112.06	116.16	120.12	124.12	128.36	132.56	136.71	140.81
Range	0.42	0.52	0.46	0.50	0.47	0.30	0.25	0.12

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.04

Maximum Std. Dev. = 0.12

Table 5. Continued.

a. First injection. (TH01 locus)

Sample Info	TH01 1	TH01 2	TH01 3	TH01 4	TH01 5	TH01 6	TH01 7	TH01 8	TH01 9	TH01 10
Ladder_a1	155.33	159.51	163.53	167.53	171.52	175.51	178.53	179.49	183.46	194.31
Ladder_b1	155.36	159.51	163.54	167.62	171.54	175.53	178.56	179.59	183.49	194.25
Ladder_c1	155.32	159.51	163.61	167.53	171.52	175.51	178.61	179.41	183.38	194.24
Ladder_d1	155.37	159.52	163.50	167.54	171.57	175.52	178.60	179.46	183.40	194.30
Ladder_e1	155.33	159.44	163.56	167.51	171.53	175.55	178.53	179.40	183.48	194.30
Ladder_f1	155.41	159.52	163.41	167.53	171.48	175.43	178.51	179.46	183.32	194.27
Ladder_g1	155.36	159.51	163.57	167.61	171.48	175.50	178.57	179.43	183.36	194.31
Ladder_h1	155.40	159.51	163.58	167.62	171.66	175.60	178.59	179.54	183.54	194.25
Ladder_I1	155.36	159.60	163.63	167.64	171.63	175.46	178.58	179.52	183.48	194.31
Ladder_j1	155.24	159.44	163.55	167.49	171.50	175.50	178.48	179.42	183.49	194.25
Ladder_k1	155.32	159.44	163.48	167.43	171.53	175.47	178.54	179.48	183.41	194.30
Ladder_L1	155.41	159.44	163.49	167.44	171.55	175.49	178.64	179.43	183.45	194.28
Ladder_m1	155.40	159.52	163.55	167.50	171.43	175.44	178.58	179.52	183.36	194.31
Ladder_n1	155.40	159.51	163.66	167.63	171.66	175.61	178.60	179.62	183.62	194.33
Ladder_o1	155.32	159.60	163.47	167.63	171.56	175.48	178.68	179.47	183.45	194.26
Ladder_p1	155.48	159.53	163.50	167.53	171.55	175.57	178.50	179.43	183.51	194.41
Mean	155.36	159.51	163.54	167.55	171.54	175.51	178.57	179.48	183.45	194.29
Std. Dev.	0.05	0.05	0.06	0.07	0.06	0.05	0.05	0.06	0.08	0.04
Min.	155.24	159.44	163.41	167.43	171.43	175.43	178.48	179.40	183.32	194.24
Max.	155.48	159.60	163.66	167.64	171.66	175.61	178.68	179.62	183.62	194.41
Range	0.24	0.16	0.25	0.21	0.23	0.18	0.20	0.22	0.30	0.17

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.04

Maximum Std. Dev. = 0.08

Table 5. Continued.

a. First injection. (D5S818 locus)

Sample Info	D5S818 1	D5S818 2	D5S818 3	D5S818 4	D5S818 5	D5S818 6	D5S818 7	D5S818 8	D5S818 9	D5S818 10
Ladder_a1	114.02	118.00	122.09	126.16	130.36	134.53	138.68	143.15	147.75	152.01
Ladder_b1	114.11	118.10	122.14	126.22	130.35	134.61	138.76	143.20	147.67	152.02
Ladder_c1	114.15	118.13	122.16	126.24	130.44	134.54	138.84	143.12	147.76	152.17
Ladder_d1	114.03	118.04	122.10	126.05	130.28	134.42	138.68	143.12	147.85	151.99
Ladder_e1	113.90	117.92	122.06	126.10	130.26	134.56	138.76	143.21	147.85	152.06
Ladder_f1	114.14	118.08	122.15	126.26	130.35	134.57	138.76	143.13	147.77	152.06
Ladder_g1	114.11	118.14	122.13	126.23	130.39	134.59	138.76	143.22	147.79	151.99
Ladder_h1	114.14	118.15	122.21	126.24	130.47	134.51	138.84	143.17	147.77	152.00
Ladder_I1	114.09	118.09	122.21	126.29	130.35	134.53	138.84	143.05	147.71	151.99
Ladder_j1	114.08	118.09	122.15	126.25	130.40	134.52	138.84	143.14	147.80	151.97
Ladder_k1	114.13	118.07	122.05	126.23	130.38	134.51	138.76	143.18	147.70	151.98
Ladder_L1	114.11	118.05	122.10	126.13	130.29	134.50	138.76	143.04	147.85	152.14
Ladder_m1	114.09	118.11	122.18	126.29	130.45	134.58	138.84	143.26	147.87	152.06
Ladder_n1	114.13	118.09	122.16	126.20	130.52	134.58	138.84	143.13	147.79	152.08
Ladder_o1	114.09	118.16	122.21	126.29	130.43	134.61	138.76	143.22	147.80	152.14
Ladder_p1	113.70	117.68	121.74	125.82	130.06	134.40	138.84	143.20	147.82	152.11
Mean	114.06	118.06	122.12	126.19	130.36	134.54	138.79	143.16	147.78	152.05
Std. Dev.	0.12	0.12	0.11	0.12	0.11	0.06	0.06	0.06	0.06	0.06
Min.	113.70	117.68	121.74	125.82	130.06	134.40	138.68	143.04	147.67	151.97
Max.	114.15	118.16	122.21	126.29	130.52	134.61	138.84	143.26	147.87	152.17
Range	0.45	0.48	0.47	0.47	0.46	0.21	0.16	0.22	0.20	0.20

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.06

Maximum Std. Dev. = 0.12

Table 5. Continued.

a. First injection. (D13S317 locus)

Sample Info	D13S317 1	D13S317 2	D13S317 3	D13S317 4	D13S317 5	D13S317 6	D13S317 7	D13S317 8
Ladder_a1	178.37	182.43	186.39	190.20	194.16	198.19	202.08	206.08
Ladder_b1	178.40	182.38	186.42	190.22	194.25	198.11	202.16	206.02
Ladder_c1	178.37	182.43	186.40	190.20	194.31	198.19	202.08	206.08
Ladder_d1	178.52	182.37	186.30	190.22	194.22	198.21	202.13	206.18
Ladder_e1	178.38	182.38	186.31	190.15	194.14	198.20	202.13	206.09
Ladder_f1	178.35	182.38	186.32	190.18	194.19	198.19	202.14	206.11
Ladder_g1	178.41	182.41	186.26	190.25	194.23	198.13	202.13	206.09
Ladder_h1	178.44	182.52	186.36	190.27	194.17	198.21	202.06	206.10
Ladder_I1	178.43	182.39	186.35	190.22	194.23	198.16	202.11	206.11
Ladder_j1	178.40	182.32	186.38	190.20	194.17	198.21	202.12	206.14
Ladder_k1	178.38	182.31	186.31	190.23	194.22	198.28	202.21	206.16
Ladder_L1	178.41	182.34	186.35	190.20	194.13	198.20	202.13	206.10
Ladder_m1	178.34	182.34	186.34	190.17	194.15	198.13	202.05	206.01
Ladder_n1	178.52	182.45	186.36	190.27	194.25	198.22	202.13	206.18
Ladder_o1	178.37	182.35	186.25	190.14	194.19	198.14	202.12	206.14
Ladder_p1	178.43	182.35	186.28	190.27	194.26	198.24	202.17	206.12
Mean	178.41	182.38	186.34	190.21	194.20	198.19	202.12	206.11
Std. Dev.	0.05	0.05	0.05	0.04	0.05	0.04	0.04	0.05
Min.	178.34	182.31	186.25	190.14	194.13	198.11	202.05	206.01
Max.	178.52	182.52	186.42	190.27	194.31	198.28	202.21	206.18
Range	0.18	0.21	0.17	0.13	0.18	0.17	0.16	0.17

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.04

Maximum Std. Dev. = 0.05

Table 5. Continued.

a. First injection. (vWA locus)

Sample Info	vWA 1	vWA 2	vWA 3	vWA 4	vWA 5	vWA 6	vWA 7
Ladder_a1	136.76	141.08	145.58	150.25	154.34	158.53	162.65
Ladder_b1	136.84	141.06	145.52	150.17	154.36	158.60	162.65
Ladder_c1	136.84	141.14	145.61	150.17	154.33	158.53	162.65
Ladder_d1	136.78	140.97	145.53	150.17	154.38	158.54	162.70
Ladder_e1	136.77	141.15	145.62	150.25	154.35	158.56	162.69
Ladder_f1	136.86	141.06	145.62	150.25	154.35	158.56	162.62
Ladder_g1	136.79	141.11	145.51	150.17	154.29	158.54	162.62
Ladder_h1	136.87	141.04	145.55	150.25	154.33	158.61	162.78
Ladder_I1	136.88	141.02	145.59	150.17	154.38	158.54	162.68
Ladder_j1	136.87	141.11	145.59	150.25	154.34	158.47	162.68
Ladder_k1	136.79	141.05	145.56	150.17	154.26	158.48	162.61
Ladder_L1	136.78	141.06	145.62	150.25	154.35	158.56	162.61
Ladder_m1	136.86	141.13	145.65	150.25	154.35	158.56	162.69
Ladder_n1	136.86	141.11	145.58	150.17	154.32	158.61	162.71
Ladder_o1	136.88	141.11	145.59	150.25	154.34	158.55	162.68
Ladder_p1	136.73	141.10	145.55	150.24	154.44	158.58	162.72
Mean	136.82	141.08	145.58	150.21	154.34	158.55	162.67
Std. Dev.	0.05	0.05	0.04	0.04	0.04	0.04	0.05
Min.	136.73	140.97	145.51	150.17	154.26	158.47	162.61
Max.	136.88	141.15	145.65	150.25	154.44	158.61	162.78
Range	0.15	0.18	0.14	0.08	0.18	0.14	0.17

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Table 5. Continued.

a. First injection. (vWA locus, continued)

Sample Info	vWA 8	vWA 9	vWA 10	vWA 11	vWA 12	vWA 13	vWA 14
Ladder_a1	166.73	170.80	174.79	178.77	182.74	186.63	190.91
Ladder_b1	166.74	170.74	174.82	178.80	182.85	186.66	190.93
Ladder_c1	166.73	170.72	174.79	178.85	182.74	186.63	190.91
Ladder_d1	166.75	170.78	174.89	178.83	182.77	186.70	190.93
Ladder_e1	166.72	170.74	174.76	178.77	182.70	186.62	190.93
Ladder_f1	166.66	170.77	174.72	178.75	182.77	186.55	190.88
Ladder_g1	166.74	170.77	174.79	178.80	182.73	186.65	190.87
Ladder_h1	166.83	170.87	174.89	178.91	182.83	186.75	190.97
Ladder_I1	166.77	170.77	174.84	178.89	182.78	186.66	190.91
Ladder_j1	166.70	170.71	174.72	178.79	182.71	186.61	190.90
Ladder_k1	166.64	170.67	174.76	178.77	182.70	186.63	190.86
Ladder_L1	166.65	170.76	174.78	178.72	182.74	186.67	190.91
Ladder_m1	166.71	170.72	174.73	178.82	182.66	186.57	190.87
Ladder_n1	166.75	170.79	174.90	178.91	182.84	186.68	190.97
Ladder_o1	166.77	170.70	174.77	178.84	182.74	186.72	190.92
Ladder_p1	166.75	170.78	174.80	178.81	182.82	186.66	190.96
Mean	166.73	170.76	174.80	178.81	182.76	186.65	190.91
Std. Dev.	0.05	0.05	0.06	0.06	0.06	0.05	0.03
Min.	166.64	170.67	174.72	178.72	182.66	186.55	190.86
Max.	166.83	170.87	174.90	178.91	182.85	186.75	190.97
Range	0.19	0.20	0.18	0.19	0.19	0.20	0.11

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.03

Maximum Std. Dev. = 0.06

Table 5. Continued.

a. First injection. (D8S1179 locus)

Sample Info	D8S1179 1	D8S1179 2	D8S1179 3	D8S1179 4	D8S1179 5	D8S1179 6	D8S1179 7	D8S1179 8	D8S1179 9	D8S1179 10	D8S1179 11	D8S1179 12
Ladder_a1	201.76	205.68	209.70	213.65	217.54	221.52	225.52	229.54	233.48	237.45	241.51	245.59
Ladder_b1	201.68	205.62	209.57	213.70	217.60	221.59	225.52	229.55	233.51	237.48	241.55	245.56
Ladder_c1	201.76	205.68	209.70	213.65	217.62	221.52	225.52	229.53	233.56	237.52	241.49	245.57
Ladder_d1	201.74	205.70	209.68	213.59	217.59	221.61	225.57	229.53	233.52	237.51	241.53	245.56
Ladder_e1	201.74	205.69	209.66	213.64	217.64	221.58	225.52	229.57	233.54	237.54	241.54	245.56
Ladder_f1	201.74	205.71	209.62	213.62	217.55	221.58	225.55	229.52	233.52	237.53	241.55	245.59
Ladder_g1	201.74	205.70	209.67	213.58	217.58	221.59	225.55	229.51	233.49	237.57	241.50	245.52
Ladder_h1	201.74	205.70	209.68	213.67	217.60	221.55	225.50	229.48	233.55	237.47	241.58	245.53
Ladder_I1	201.72	205.64	209.58	213.53	217.57	221.47	225.46	229.47	233.41	237.45	241.42	245.48
Ladder_j1	201.73	205.75	209.70	213.66	217.64	221.64	225.56	229.59	233.54	237.59	241.57	245.65
Ladder_k1	201.73	205.69	209.65	213.71	217.62	221.63	225.57	229.52	233.57	237.55	241.54	245.55
Ladder_L1	201.74	205.70	209.75	213.66	217.67	221.60	225.63	229.60	233.58	237.49	241.58	245.68
Ladder_m1	201.66	205.62	209.59	213.58	217.58	221.52	225.47	229.51	233.49	237.49	241.50	245.52
Ladder_n1	201.74	205.78	209.68	213.67	217.67	221.61	225.57	229.54	233.60	237.60	241.53	245.64
Ladder_o1	201.81	205.74	209.61	213.66	217.72	221.63	225.64	229.58	233.62	237.59	241.57	245.65
Ladder_p1	201.78	205.74	209.71	213.69	217.69	221.62	225.57	229.61	233.58	237.65	241.58	245.68
Mean	201.74	205.70	209.66	213.64	217.62	221.58	225.55	229.54	233.54	237.53	241.53	245.58
Std. Dev.	0.03	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.06	0.04	0.06
Min.	201.66	205.62	209.57	213.53	217.54	221.47	225.46	229.47	233.41	237.45	241.42	245.48
Max.	201.81	205.78	209.75	213.71	217.72	221.64	225.64	229.61	233.62	237.65	241.58	245.68
Range	0.15	0.16	0.18	0.18	0.18	0.17	0.18	0.14	0.21	0.20	0.16	0.20

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.03

Maximum Std. Dev. = 0.06

Table 5. Continued.

b. Second injection. (D3S1358 locus)

Sample Info	D3S1358 1	D3S1358 2	D3S1358 3	D3S1358 4	D3S1358 5	D3S1358 6	D3S1358 7	D3S1358 8
Ladder_a2	111.95	116.00	120.02	124.09	128.21	132.37	136.59	140.73
Ladder_b2	111.93	116.15	120.11	124.04	128.25	132.43	136.58	140.82
Ladder_c2	111.89	116.03	119.98	123.98	128.18	132.43	136.50	140.73
Ladder_d2	111.92	116.08	120.00	124.03	128.20	132.49	136.52	140.73
Ladder_e2	111.96	116.13	120.05	124.08	128.32	132.52	136.54	140.79
Ladder_f2	111.96	116.04	120.02	123.97	128.13	132.34	136.45	140.73
Ladder_g2	111.93	116.10	120.02	124.05	128.29	132.50	136.61	140.89
Ladder_h2	112.06	116.14	120.05	124.08	128.31	132.44	136.61	140.80
Ladder_I2	111.91	116.07	120.06	124.09	128.24	132.52	136.62	140.79
Ladder_j2	111.96	116.13	120.05	124.08	128.24	132.44	136.54	140.79
Ladder_k2	111.83	116.08	120.08	123.97	128.21	132.43	136.53	140.78
Ladder_L2	111.96	115.99	120.06	124.03	128.20	132.41	136.53	140.72
Ladder_m2	111.99	116.01	120.00	124.03	128.20	132.41	136.52	140.73
Ladder_n2	111.96	116.06	120.06	124.10	128.27	132.49	136.61	140.80
Ladder_o2	111.68	115.69	119.56	123.67	127.86	132.23	136.44	140.78
Ladder_p2	111.54	115.64	119.54	123.60	127.83	132.17	136.53	140.88
Mean	111.90	116.02	119.98	123.99	128.18	132.41	136.55	140.78
Std. Dev.	0.13	0.15	0.17	0.15	0.14	0.10	0.06	0.05
Min.	111.54	115.64	119.54	123.60	127.83	132.17	136.44	140.72
Max.	112.06	116.15	120.11	124.10	128.32	132.52	136.62	140.89
Range	0.52	0.51	0.57	0.50	0.49	0.35	0.18	0.17

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.05

Maximum Std. Dev. = 0.17

Table 5. Continued.

b. Second injection. (TH01 locus)

Sample Info	TH01 1	TH01 2	TH01 3	TH01 4	TH01 5	TH01 6	TH01 7	TH01 8	TH01 9	TH01 10
Ladder_a1	155.28	159.51	163.47	167.58	171.59	175.52	178.65	179.45	183.45	194.36
Ladder_b1	155.33	159.51	163.46	167.57	171.50	175.44	178.56	179.45	183.29	194.25
Ladder_c1	155.28	159.51	163.55	167.50	171.51	175.52	178.49	179.53	183.37	194.28
Ladder_d1	155.37	159.44	163.43	167.50	171.48	175.45	178.39	179.34	183.39	194.23
Ladder_e1	155.40	159.51	163.59	167.50	171.55	175.51	178.52	179.39	183.34	194.20
Ladder_f1	155.29	159.44	163.43	167.50	171.40	175.45	178.55	179.42	183.39	194.23
Ladder_g1	155.45	159.52	163.50	167.55	171.60	175.56	178.57	179.44	183.47	194.42
Ladder_h1	155.37	159.52	163.59	167.56	171.54	175.51	178.52	179.47	183.51	194.33
Ladder_I1	155.32	159.44	163.48	167.44	171.55	175.41	178.41	179.43	183.37	194.28
Ladder_j1	155.32	159.43	163.51	167.58	171.55	175.60	178.53	179.48	183.43	194.28
Ladder_k1	155.36	159.51	163.53	167.60	171.58	175.63	178.56	179.59	183.54	194.30
Ladder_L1	155.32	159.43	163.44	167.60	171.50	175.48	178.50	179.46	183.42	194.33
Ladder_m1	155.41	159.59	163.60	167.58	171.56	175.54	178.47	179.58	183.46	194.42
Ladder_n1	155.32	159.43	163.52	167.60	171.58	175.56	178.58	179.46	183.46	194.25
Ladder_o1	155.32	159.43	163.59	167.64	171.60	175.55	178.71	179.65	183.51	194.31
Ladder_p1	155.41	159.53	163.51	167.63	171.52	175.63	178.58	179.51	183.39	194.37
Mean	155.35	159.48	163.51	167.56	171.54	175.52	178.54	179.48	183.42	194.30
Std. Dev.	0.05	0.05	0.06	0.06	0.05	0.07	0.08	0.08	0.07	0.07
Min.	155.28	159.43	163.43	167.44	171.40	175.41	178.39	179.34	183.29	194.20
Max.	155.45	159.59	163.60	167.64	171.60	175.63	178.71	179.65	183.54	194.42
Range	0.17	0.16	0.17	0.20	0.20	0.22	0.32	0.31	0.25	0.22

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.05

Maximum Std. Dev. = 0.08

Table 5. Continued.

b. Second injection. (D5S818 locus)

Sample Info	D5S818 1	D5S818 2	D5S818 3	D5S818 4	D5S818 5	D5S818 6	D5S818 7	D5S818 8	D5S818 9	D5S818 10
Ladder_a1	113.97	118.12	122.16	126.18	130.40	134.51	138.84	143.15	147.66	152.02
Ladder_b1	114.11	118.05	122.11	126.22	130.45	134.58	138.76	143.24	147.83	152.01
Ladder_c1	113.99	118.00	122.13	126.23	130.38	134.50	138.76	143.15	147.74	152.02
Ladder_d1	114.07	118.03	122.05	126.19	130.38	134.54	138.76	143.25	147.84	152.08
Ladder_e1	114.12	118.16	122.17	126.31	130.41	134.64	138.84	143.25	147.69	152.00
Ladder_f1	114.03	117.99	122.07	126.12	130.31	134.55	138.68	143.07	147.66	151.99
Ladder_g1	114.09	118.13	122.22	126.28	130.47	134.55	138.92	143.21	147.76	152.07
Ladder_h1	114.13	118.17	122.17	126.30	130.41	134.72	138.84	143.21	147.76	152.24
Ladder_I1	114.06	118.10	122.18	126.23	130.41	134.56	138.84	143.18	147.78	152.06
Ladder_j1	114.12	118.09	122.17	126.23	130.49	134.56	138.76	143.17	147.69	152.00
Ladder_k1	113.91	118.04	122.14	126.13	130.39	134.55	138.68	143.16	147.60	151.93
Ladder_L1	114.04	118.09	122.11	126.18	130.38	134.46	138.76	143.12	147.67	152.01
Ladder_m1	114.07	118.11	122.12	126.19	130.38	134.62	138.84	143.15	147.74	152.18
Ladder_n1	114.04	118.09	122.11	126.18	130.46	134.62	138.92	143.20	147.76	152.01
Ladder_o1	113.71	117.77	121.76	125.83	130.07	134.41	138.75	143.25	147.69	152.17
Ladder_p1	113.61	117.54	121.63	125.74	130.03	134.42	138.75	143.36	147.96	152.11
Mean	114.00	118.03	122.08	126.16	130.36	134.55	138.79	143.20	147.74	152.06
Std. Dev.	0.15	0.16	0.16	0.16	0.13	0.08	0.07	0.07	0.09	0.08
Min.	113.61	117.54	121.63	125.74	130.03	134.41	138.68	143.07	147.60	151.93
Max.	114.13	118.17	122.22	126.31	130.49	134.72	138.92	143.36	147.96	152.24
Range	0.52	0.63	0.59	0.57	0.46	0.31	0.24	0.29	0.36	0.31

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.07

Maximum Std. Dev. = 0.16

Table 5. Continued.

b. Second injection. (D13S317 locus)

Sample Info	D13S317 1	D13S317 2	D13S317 3	D13S317 4	D13S317 5	D13S317 6	D13S317 7	D13S317 8
Ladder_a1	178.41	182.41	186.40	190.22	194.28	198.18	202.09	206.13
Ladder_b1	178.40	182.25	186.33	190.10	194.17	198.17	202.10	205.98
Ladder_c1	178.41	182.41	186.32	190.30	194.20	198.18	202.01	206.13
Ladder_d1	178.31	182.28	186.24	190.12	194.08	198.11	202.07	206.07
Ladder_e1	178.36	182.39	186.34	190.12	194.12	198.04	201.99	206.14
Ladder_f1	178.31	182.36	186.16	190.12	194.31	198.19	202.16	206.08
Ladder_g1	178.49	182.36	186.31	190.17	194.19	198.27	202.07	206.06
Ladder_h1	178.44	182.40	186.27	190.14	194.17	198.19	202.15	206.14
Ladder_I1	178.33	182.34	186.35	190.20	194.20	198.20	202.14	206.11
Ladder_j1	178.45	182.40	186.34	190.20	194.28	198.20	202.14	206.05
Ladder_k1	178.48	182.51	186.53	190.38	194.38	198.28	202.15	206.21
Ladder_L1	178.34	182.39	186.35	190.22	194.17	198.11	202.08	206.09
Ladder_m1	178.55	182.43	186.38	190.17	194.26	198.20	202.08	206.08
Ladder_n1	178.42	182.47	186.43	190.22	194.25	198.19	202.08	206.08
Ladder_o1	178.55	182.48	186.49	190.25	194.39	198.29	202.14	206.19
Ladder_p1	178.35	182.54	186.41	190.20	194.21	198.23	202.10	206.01
Mean	178.41	182.40	186.35	190.20	194.23	198.19	202.10	206.10
Std. Dev.	0.08	0.08	0.09	0.07	0.09	0.06	0.05	0.06
Min.	178.31	182.25	186.16	190.10	194.08	198.04	201.99	205.98
Max.	178.55	182.54	186.53	190.38	194.39	198.29	202.16	206.21
Range	0.24	0.29	0.37	0.28	0.31	0.25	0.17	0.23

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.05

Maximum Std. Dev. = 0.09

Table 5. Continued.

b. Second injection. (vWA locus)

Sample Info	vWA 1	vWA 2	vWA 3	vWA 4	vWA 5	vWA 6	vWA 7
Ladder_a1	136.83	141.07	145.57	150.17	154.28	158.52	162.66
Ladder_b1	136.82	141.08	145.58	150.25	154.33	158.53	162.58
Ladder_c1	136.74	141.07	145.58	150.17	154.37	158.52	162.66
Ladder_d1	136.76	141.08	145.58	150.17	154.30	158.55	162.63
Ladder_e1	136.78	141.13	145.55	150.17	154.33	158.53	162.64
Ladder_f1	136.77	141.08	145.50	150.17	154.30	158.47	162.55
Ladder_g1	136.85	141.06	145.61	150.25	154.38	158.54	162.71
Ladder_h1	136.85	141.15	145.61	150.25	154.38	158.54	162.71
Ladder_I1	136.85	141.13	145.65	150.25	154.35	158.56	162.61
Ladder_j1	136.78	141.13	145.55	150.17	154.33	158.53	162.64
Ladder_k1	136.77	141.04	145.55	150.17	154.36	158.60	162.73
Ladder_L1	136.77	141.06	145.52	150.17	154.33	158.53	162.64
Ladder_m1	136.76	141.08	145.58	150.25	154.42	158.61	162.72
Ladder_n1	136.84	141.06	145.61	150.17	154.33	158.53	162.72
Ladder_o1	136.69	141.12	145.55	150.17	154.41	158.61	162.79
Ladder_p1	136.70	141.14	145.66	150.41	154.53	158.66	162.65
Mean	136.79	141.09	145.58	150.21	154.36	158.55	162.67
Std. Dev.	0.05	0.03	0.04	0.07	0.06	0.05	0.06
Min.	136.69	141.04	145.50	150.17	154.28	158.47	162.55
Max.	136.85	141.15	145.66	150.41	154.53	158.66	162.79
Range	0.16	0.11	0.16	0.24	0.25	0.19	0.24

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Table 5. Continued.

b. Second injection. (vWA locus, continued)

Sample Info	vWA 8	vWA 9	vWA 10	vWA 11	vWA 12	vWA 13	vWA 14
Ladder_a1	166.69	170.71	174.72	178.81	182.81	186.72	190.94
Ladder_b1	166.68	170.62	174.71	178.72	182.73	186.57	190.81
Ladder_c1	166.69	170.71	174.72	178.81	182.73	186.64	190.86
Ladder_d1	166.62	170.68	174.74	178.71	182.67	186.56	190.83
Ladder_e1	166.70	170.75	174.72	178.76	182.71	186.65	190.90
Ladder_f1	166.70	170.76	174.74	178.71	182.67	186.56	190.91
Ladder_g1	166.76	170.73	174.77	178.81	182.76	186.62	190.88
Ladder_h1	166.69	170.74	174.79	178.76	182.71	186.67	190.86
Ladder_I1	166.65	170.68	174.78	178.80	182.74	186.67	190.83
Ladder_j1	166.70	170.76	174.81	178.85	182.80	186.66	190.99
Ladder_k1	166.80	170.87	174.84	178.88	182.83	186.77	191.01
Ladder_L1	166.72	170.71	174.77	178.82	182.71	186.67	190.94
Ladder_m1	166.79	170.85	174.82	178.79	182.75	186.70	190.88
Ladder_n1	166.72	170.79	174.77	178.82	182.79	186.67	190.94
Ladder_o1	166.76	170.81	174.84	178.86	182.88	186.72	190.95
Ladder_p1	166.78	170.82	174.86	178.89	182.77	186.64	190.89
Mean	166.72	170.75	174.78	178.80	182.75	186.66	190.90
Std. Dev.	0.05	0.07	0.05	0.06	0.06	0.06	0.06
Min.	166.62	170.62	174.71	178.71	182.67	186.56	190.81
Max.	166.80	170.87	174.86	178.89	182.88	186.77	191.01
Range	0.18	0.25	0.15	0.18	0.21	0.21	0.20

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.03

Maximum Std. Dev. = 0.07

Table 5. Continued.

b. Second injection. (D8S1179 locus)

Sample Info	D8S1179 1	D8S1179 2	D8S1179 3	D8S1179 4	D8S1179 5	D8S1179 6	D8S1179 7	D8S1179 8	D8S1179 9	D8S1179 10	D8S1179 11	D8S1179 12
Ladder_a1	201.69	205.64	209.61	213.59	217.59	221.52	225.47	229.51	233.49	237.48	241.49	245.51
Ladder_b1	201.70	205.66	209.64	213.55	217.64	221.58	225.46	229.51	233.50	237.50	241.51	245.55
Ladder_c1	201.69	205.65	209.61	213.60	217.52	221.53	225.48	229.44	233.42	237.42	241.51	245.45
Ladder_d1	201.67	205.67	209.60	213.63	217.59	221.48	225.39	229.40	233.42	237.46	241.43	245.49
Ladder_e1	201.67	205.58	209.59	213.61	217.56	221.53	225.51	229.43	233.52	237.47	241.51	245.49
Ladder_f1	201.68	205.68	209.70	213.65	217.61	221.59	225.51	229.52	233.54	237.49	241.63	245.62
Ladder_g1	201.75	205.66	209.66	213.60	217.63	221.51	225.50	229.49	233.42	237.45	241.49	245.55
Ladder_h1	201.75	205.66	209.67	213.61	217.65	221.54	225.45	229.45	233.47	237.50	241.55	245.54
Ladder_I1	201.74	205.71	209.69	213.60	217.61	221.63	225.51	229.56	233.54	237.54	241.55	245.58
Ladder_J1	201.75	205.73	209.64	213.65	217.59	221.55	225.52	229.50	233.50	237.51	241.54	245.58
Ladder_k1	201.75	205.73	209.65	213.74	217.60	221.56	225.54	229.52	233.53	237.54	241.57	245.62
Ladder_L1	201.68	205.68	209.62	213.58	217.62	221.61	225.52	229.53	233.47	237.51	241.57	245.55
Ladder_m1	201.68	205.68	209.61	213.64	217.60	221.58	225.49	229.50	233.52	237.48	241.53	245.52
Ladder_n1	201.76	205.76	209.69	213.64	217.60	221.58	225.49	229.50	233.52	237.56	241.53	245.51
Ladder_o1	201.74	205.79	209.77	213.69	217.70	221.64	225.60	229.58	233.56	237.57	241.58	245.53
Ladder_p1	201.79	205.77	209.77	213.70	217.65	221.61	225.58	229.57	233.58	237.52	241.55	245.60
Mean	201.72	205.69	209.66	213.63	217.61	221.57	225.50	229.50	233.50	237.50	241.53	245.54
Std. Dev.	0.04	0.05	0.06	0.05	0.04	0.05	0.05	0.05	0.05	0.04	0.05	0.05
Min.	201.67	205.58	209.59	213.55	217.52	221.48	225.39	229.40	233.42	237.42	241.43	245.45
Max.	201.79	205.79	209.77	213.74	217.70	221.64	225.60	229.58	233.58	237.57	241.63	245.62
Range	0.12	0.21	0.18	0.19	0.18	0.16	0.21	0.18	0.16	0.15	0.20	0.17

Std. Dev.: standard deviation, Min.: minimum, Max.: maximum.

Minimum Std. Dev. = 0.04

Maximum Std. Dev. = 0.06

Table 6. BodePlex 3 reproducibility. Genotypes of the 16 9947A samples amplified with BodePlex 3. Data represents two separate injections.

Sample Info	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2
9947_a1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_a2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_b1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_b2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_c1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_c2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_d1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_d2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_e1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_e2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_f1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_f2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_g1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_g2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_h1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_h2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_I1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_I2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_j1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_j2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_k1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_k2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_L1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_L2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_m1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_m2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_n1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_n2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_o1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_o2	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_p1	14	15	8	9.3	11	11	11	11	17	18	13	13
9947_p2	14	15	8	9.3	11	11	11	11	17	18	13	13

Table 7. Species DNA samples. Two different template amounts for each species DNA were amplified with BodePlex 3.

Species	Amount #1 (ng)	Amount #2 (ng)
Beaver	0.25	5
Bovine	0.25	5
C. albicans	0.25	5
Chicken	0.25	5
Cottontail rabbit	0.25	5
Coyote	0.25	5
Dog	0.25	5
E. coli	0.25	5
Mink	0.25	5
Monkey	0.25	5
Mouse	0.25	5
Pig	0.25	5
Rabbit	0.25	5
Rat	0.25	5
Salmon	0.25	5

Table 8. Species samples that produced peaks when amplified with BodePlex 3.

Species	DNA amount (ng)	Base pair size	Peak height (RFU)	Locus/allele equivalent	Dye color
Chicken	0.25	130.49	56	D5S818 (11)	Green
E. coli	5	182.67	59	TH01 (OL)	Blue
Monkey	0.25	241.51	425	None	Green
		245.57	935	None	Green
Monkey	5	149.57	181	D3S1358 (OL)	Blue
		241.36	9838	None	Green
		245.49	9278	None	Green
Mouse	5	128.42	104	D3S1358 (16)	Blue
Pig	5	236.44	69	None	Green
Rabbit	5	112.83	217	D3S1358 (OL)	Blue
Salmon	5	154.49	150	None	Blue

RFU: relative fluorescence unit, OL: off-ladder allele

Table 9. BodePlex 3 sensitivity - 9948 samples. Ten different 9948 DNA template amounts were amplified with BodePlex 3 in triplicate. Average peak height values at each locus were calculated and reported in RFU. The total average peak height value (Total av. value) represents all loci. Highlighted cells denote allelic drop out at heterozygote loci.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0ng	0	0	0	0	0	0	
9948b_0ng	0	0	0	0	0	0	
9948c_0ng	0	0	0	0	0	0	Total av. value
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	0.00	0.00	0.00	0.00	0.00	0.00	
Range	0.00	0.00	0.00	0.00	0.00	0.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.005ng	55	0	72	105	52	0	
9948b_0.005ng	0	0	81	88	123	115.5	
9948c_0.005ng	71	56	119	0	131	0	Total av. value
Mean	42.00	18.67	90.67	64.33	102.00	38.50	59.36
Min.	0.00	0.00	72.00	0.00	52.00	0.00	
Max.	71.00	56.00	119.00	105.00	131.00	115.50	
Range	71.00	56.00	47.00	105.00	79.00	115.50	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.01ng	0	0	0	0	0	0	
9948b_0.01ng	140.5	84	59	144	195	76.5	
9948c_0.01ng	124.5	0	153.5	138	74	0	Total av. value
Mean	88.33	28.00	70.83	94.00	89.67	25.50	66.06
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	140.50	84.00	153.50	144.00	195.00	76.50	
Range	140.50	84.00	153.50	144.00	195.00	76.50	

Table 9. Continued.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.025ng	333.5	212	361.5	521	406	231	
9948b_0.025ng	359.5	120.5	272	280	362	113.5	
9948c_0.025ng	399	183	314	457	445	130	Total av. value
Mean	364.00	171.83	315.83	419.33	404.33	158.17	305.58
Min.	333.50	120.50	272.00	280.00	362.00	113.50	
Max.	399.00	212.00	361.50	521.00	445.00	231.00	
Range	65.50	91.50	89.50	241.00	83.00	117.50	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.05ng	0	0	0	0	0	0	
9948b_0.05ng	458.5	172.5	431	886	871	286	
9948c_0.05ng	0	0	0	0	0	0	Total av. value
Mean	152.83	57.50	143.67	295.33	290.33	95.33	172.50
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	458.50	172.50	431.00	886.00	871.00	286.00	
Range	458.50	172.50	431.00	886.00	871.00	286.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.1ng	420	231	412	566	591	231	
9948b_0.1ng	1308	434	973	1313	1400	422	
9948c_0.1ng	801	383	1003	1393	1345	510.5	Total av. value
Mean	843.00	349.33	796.00	1090.67	1112.00	387.83	763.14
Min.	420.00	231.00	412.00	566.00	591.00	231.00	
Max.	1308.00	434.00	1003.00	1393.00	1400.00	510.50	
Range	888.00	203.00	591.00	827.00	809.00	279.50	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.25ng	2883.5	1220.5	2375.5	3281	4110	1902.5	
9948b_0.25ng	3008	1050.5	2631	3585	3317	2153.5	
9948c_0.25ng	2482.5	1059	2100	3256	3150	1320.5	Total av. value
Mean	2791.33	1110.00	2368.83	3374.00	3525.67	1792.17	2493.67
Min.	2482.50	1050.50	2100.00	3256.00	3150.00	1320.50	
Max.	3008.00	1220.50	2631.00	3585.00	4110.00	2153.50	
Range	525.50	170.00	531.00	329.00	960.00	833.00	

Table 9. Continued.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_0.5ng	4925	1504	4037.5	6483	6159	2602.5	
9948b_0.5ng	4705.5	2128.5	3630	5875	5533	2381.5	
9948c_0.5ng	4953.5	1774.5	4126	5432	5842	2216.5	Total av. value
Mean	4861.33	1802.33	3931.17	5930.00	5844.67	2400.17	4128.28
Min.	4705.50	1504.00	3630.00	5432.00	5533.00	2216.50	
Max.	4953.50	2128.50	4126.00	6483.00	6159.00	2602.50	
Range	248.00	624.50	496.00	1051.00	626.00	386.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_1ng	7895.5	3949.5	7876	9551	7318	4977.5	
9948b_1ng	7976	4486.5	9445	9456	7342	5818	
9948c_1ng	8045	3936	8890	9693	7439	5151.5	Total av. value
Mean	7972.17	4124.00	8737.00	9566.67	7366.33	5315.67	7180.31
Min.	7895.50	3936.00	7876.00	9456.00	7318.00	4977.50	
Max.	8045.00	4486.50	9445.00	9693.00	7439.00	5818.00	
Range	149.50	550.50	1569.00	237.00	121.00	840.50	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9948a_2ng	7897.5	7153	8944	9192	7221	7575.5	
9948b_2ng	8207.5	7811	8720.5	9191	4581	7043.5	
9948c_2ng	8448	7682	8813	8788	5103	7235.5	Total av. value
Mean	8184.33	7548.67	8825.83	9057.00	5635.00	7284.83	7755.94
Min.	7897.50	7153.00	8720.50	8788.00	4581.00	7043.50	
Max.	8448.00	7811.00	8944.00	9192.00	7221.00	7575.50	
Range	550.50	658.00	223.50	404.00	2640.00	532.00	

Table 10. BodePlex 3 sensitivity - 9947A samples. Ten different 9947A DNA template amounts were amplified with BodePlex 3 in triplicate. Average peak height values at each locus were calculated and reported in RFU. The total average peak height value (Total av. value) represents all loci. Highlighted cells denote allelic drop out.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0ng	0	0	0	0	0	0	
9947b_0ng	0	0	0	0	0	0	
9947c_0ng	0	0	0	0	0	0	Total av. value
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	0.00	0.00	0.00	0.00	0.00	0.00	
Range	0.00	0.00	0.00	0.00	0.00	0.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.005ng	0	0	0	0	0	0	
9947b_0.005ng	0	0	81	0	0	0	
9947c_0.005ng	0	0	0	53	0	0	Total av. value
Mean	0.00	0.00	27.00	17.67	0.00	0.00	7.44
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	0.00	0.00	81.00	53.00	0.00	0.00	
Range	0.00	0.00	81.00	53.00	0.00	0.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.01ng	0	0	0	0	0	0	
9947b_0.01ng	0	0	85	0	51	0	
9947c_0.01ng	58	0	78	0	79.5	61	Total av. value
Mean	19.33	0.00	54.33	0.00	43.50	20.33	22.92
Min.	0.00	0.00	0.00	0.00	0.00	0.00	
Max.	58.00	0.00	85.00	0.00	79.50	61.00	
Range	58.00	0.00	85.00	0.00	79.50	61.00	

Table 10. Continued.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.025ng	119.5	0	269	177	0	132	
9947b_0.025ng	84	0	94	70	0	96	
9947c_0.025ng	123	0	177	57	64	81	Total av. value
Mean	108.83	0.00	180.00	101.33	21.33	103.00	85.75
Min.	84.00	0.00	94.00	57.00	0.00	81.00	
Max.	123.00	0.00	269.00	177.00	64.00	132.00	
Range	39.00	0.00	175.00	120.00	64.00	51.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.05ng	194	0	218	146	83	205	
9947b_0.05ng	346.5	0	403	382	99	401	
9947c_0.05ng	138.5	54	371	262	103	344	Total av. value
Mean	226.33	18.00	330.67	263.33	95.00	316.67	208.33
Min.	138.50	0.00	218.00	146.00	83.00	205.00	
Max.	346.50	54.00	403.00	382.00	103.00	401.00	
Range	208.00	54.00	185.00	236.00	20.00	196.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.1ng	310.5	66	791	380	182.5	323	
9947b_0.1ng	359.5	68	642	390	208	369	
9947c_0.1ng	212.5	50	451	441	203	358	Total av. value
Mean	294.17	61.33	628.00	403.67	197.83	350.00	322.50
Min.	212.50	50.00	451.00	380.00	182.50	323.00	
Max.	359.50	68.00	791.00	441.00	208.00	369.00	
Range	147.00	18.00	340.00	61.00	25.50	46.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.25ng	1476.5	205.5	2848	1995	850	1750	
9947b_0.25ng	653.5	179	1746	1369	475	1087	
9947c_0.25ng	830.5	82.5	1593	1786	452.5	1177	Total av. value
Mean	986.83	155.67	2062.33	1716.67	592.50	1338.00	1142.00
Min.	653.50	82.50	1593.00	1369.00	452.50	1087.00	
Max.	1476.50	205.50	2848.00	1995.00	850.00	1750.00	
Range	823.00	123.00	1255.00	626.00	397.50	663.00	

Table 10. Continued.

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_0.5ng	2023.5	347	3713	3749	1419	3407	
9947b_0.5ng	1645.5	229.5	3715	3260	1168.5	2491	
9947c_0.5ng	1704	213.5	3093	3013	1154.5	2093	Total av. value
Mean	1791.00	263.33	3507.00	3340.67	1247.33	2663.67	2135.50
Min.	1645.50	213.50	3093.00	3013.00	1154.50	2093.00	
Max.	2023.50	347.00	3715.00	3749.00	1419.00	3407.00	
Range	378.00	133.50	622.00	736.00	264.50	1314.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_1ng	3656.5	598.5	7519	6030	2254	4618	
9947b_1ng	3470.5	736.5	6841	5275	1963	4568	
9947c_1ng	3245.5	616	5655	5302	1634.5	4318	Total av. value
Mean	3457.50	650.33	6671.67	5535.67	1950.50	4501.33	3794.50
Min.	3245.50	598.50	5655.00	5275.00	1634.50	4318.00	
Max.	3656.50	736.50	7519.00	6030.00	2254.00	4618.00	
Range	411.00	138.00	1864.00	755.00	619.50	300.00	

Sample Info	D3S1358	TH01	D5S818	D13S317	vWA	D8S1179	
9947a_2ng	8584.5	1011.5	9645	9990	6089.5	7580	
9947b_2ng	7237	1150	9804	10023	4946.5	7789	
9947c_2ng	8151.5	1237	9433	9863	5697.5	7786	Total av. value
Mean	7991.00	1132.83	9627.33	9958.67	5577.83	7718.33	7001.00
Min.	7237.00	1011.50	9433.00	9863.00	4946.50	7580.00	
Max.	8584.50	1237.00	9804.00	10023.00	6089.50	7789.00	
Range	1347.50	225.50	371.00	160.00	1143.00	209.00	

Table 11. BodePlex 3 sensitivity - percent of dye pull-up. Average of percent dye pull-up (and reverse dye pull-up) was calculated for four DNA template amounts, only when the peak heights of pull-up peaks exceeded 50 RFU. The range of peak heights (Av. peak height range) in each dye color is reported in RFU values.

BodePlex 3 Color	BLUE		GREEN		YELLOW	
0.25 ng						
Av. peak height range	83	3008	1369	3585	453	4110
Blue pull-up						
Green pull-up						
Yellow pull-up	No Pull-up >50 RFU		No Pull-up >50 RFU		No Pull-up >50 RFU	
Red pull-up						
0.5 ng						
Av. peak height range	214	4954	3013	6483	265	6159
Blue pull-up						
Green pull-up						
Yellow pull-up	No Pull-up >50 RFU		No Pull-up >50 RFU		No Pull-up >50 RFU	
Red pull-up						
1 ng						
Av. peak height range	599	8045	5275	9693	1635	7439
Blue pull-up						
Green pull-up	12.65%				16.30%	(reverse)
Yellow pull-up			46.52%			
Red pull-up						
2 ng						
Av. peak height range	1012	8585	8721	10023	4581	7789
Blue pull-up						
Green pull-up	37.28%				23.11%	(reverse)
Yellow pull-up			47.67%			
Red pull-up						

Table 12. BodePlex 3 sensitivity - heterozygote peak height balance. Percent of peak height balance was calculated for the different template amounts of 9947A (a) and 9948 (b) DNA, at heterozygote loci. Highlighted cells indicate % balance values below 30%.

a. Percent peak height balance for 9947A samples.

Sample Info	D3S1358 1	D3S1358 2	% Balance	TH01 1	TH01 2	% Balance	vWA 1	vWA 2	% Balance
9947a_0ng	0	0		0	0		0	0	
9947b_0ng	0	0		0	0		0	0	
9947c_0ng	0	0		0	0		0	0	
9947a_0.005ng	0	0		0	0		0	0	
9947b_0.005ng	0	0		0	0		0	0	
9947c_0.005ng	0	0		0	0		0	0	
9947a_0.01ng	0	0		0	0		0	0	
9947b_0.01ng	0	0		0	0		51	0	
9947c_0.01ng	0	58		0	0		53	106	50.00%
9947a_0.025ng	173	66	38.15%	0	0		0	0	
9947b_0.025ng	57	111	51.35%	0	0		0	0	
9947c_0.025ng	94	152	61.84%	0	0		64	0	
9947a_0.05ng	109	279	39.07%	0	0		0	83	
9947b_0.05ng	253	440	57.50%	0	0		71	127	55.91%
9947c_0.05ng	141	136	96.45%	54	0		117	89	76.07%
9947a_0.1ng	304	317	95.90%	66	0		222	143	64.41%
9947b_0.1ng	499	220	44.09%	0	68		232	184	79.31%
9947c_0.1ng	144	281	51.25%	0	50		141	265	53.21%
9947a_0.25ng	1497	1456	97.26%	220	191	86.82%	889	811	91.23%
9947b_0.25ng	591	716	82.54%	126	232	54.31%	523	427	81.64%
9947c_0.25ng	733	928	78.99%	114	51	44.74%	412	493	83.57%
9947a_0.5ng	2204	1843	83.62%	306	388	78.87%	1686	1152	68.33%
9947b_0.5ng	1699	1592	93.70%	279	180	64.52%	1152	1185	97.22%
9947c_0.5ng	1271	2137	59.48%	236	191	80.93%	1206	1103	91.46%
9947a_1ng	3618	3695	97.92%	601	596	99.17%	2427	2081	85.74%
9947b_1ng	3665	3276	89.39%	744	729	97.98%	1980	1946	98.28%
9947c_1ng	3014	3477	86.68%	661	571	86.38%	1683	1586	94.24%
9947a_2ng	8780	8389	95.55%	1054	969	91.94%	6460	5719	88.53%
9947b_2ng	7800	6674	85.56%	1182	1118	94.59%	5365	4528	84.40%
9947c_2ng	8490	7813	92.03%	1316	1158	87.99%	5879	5516	93.83%

Table 12. Continued.

b. Percent peak height balance for 9948 samples.

Sample Info	D3S1358 1	D3S1358 2	% Balance	TH01 1	TH01 2	% Balance	D5S818 1	D5S818 2	% Balance	D8S1179	D8S1179	% Balance
9948a_0ng	0	0		0	0		0	0		0	0	
9948b_0ng	0	0		0	0		0	0		0	0	
9948c_0ng	0	0		0	0		0	0		0	0	
9948a_0.005ng	55	0		0	0		0	72		0	0	
9948b_0.005ng	0	0		0	0		81	0		152	79	51.97%
9948c_0.005ng	0	71		56	0		0	119		0	0	
9948a_0.01ng	0	0		0	0		0	0		0	0	
9948b_0.01ng	80	201	39.80%	0	84		0	59		73	80	91.25%
9948c_0.01ng	98	151	64.90%	0	0		135	172	78.49%	0	0	
9948a_0.025ng	293	374	78.34%	297	127	42.76%	291	432	67.36%	277	185	66.79%
9948b_0.025ng	482	237	49.17%	148	93	62.84%	262	282	92.91%	128	99	77.34%
9948c_0.025ng	340	458	74.24%	229	137	59.83%	315	313	99.37%	130	0	
9948a_0.05ng	0	0		0	0		0	0				
9948b_0.05ng	710	207	29.15%	261	84	32.18%	456	406	89.04%	397	175	44.08%
9948c_0.05ng	0	0		0	0		0	0		0	0	
9948a_0.1ng	476	364	76.47%	227	235	96.60%	361	463	77.97%	196	266	73.68%
9948b_0.1ng	1284	1332	96.40%	627	241	38.44%	1147	799	69.66%	340	504	67.46%
9948c_0.1ng	726	876	82.88%	463	303	65.44%	913	1093	83.53%	402	619	64.94%
9948a_0.25ng	2904	2863	98.59%	1443	998	69.16%	1950	2801	69.62%	2194	1611	73.43%
9948b_0.25ng	2767	3249	85.16%	1425	676	47.44%	2313	2949	78.43%	2174	2133	98.11%
9948c_0.25ng	2683	2282	85.05%	1140	978	85.79%	2363	1837	77.74%	1148	1493	76.89%
9948a_0.5ng	4790	5060	94.66%	1436	1572	91.35%	4021	4054	99.19%	2623	2582	98.44%
9948b_0.5ng	4560	4851	94.00%	2422	1835	75.76%	3402	3858	88.18%	2451	2312	94.33%
9948c_0.5ng	5266	4641	88.13%	1557	1992	78.16%	4786	3466	72.42%	2206	2227	99.06%
9948a_1ng	7810	7981	97.86%	4118	3781	91.82%	7634	8118	94.04%	5197	4758	91.55%
9948b_1ng	7905	8047	98.24%	4327	4646	93.13%	9426	9464	99.60%	6268	5368	85.64%
9948c_1ng	7970	8120	98.15%	4017	3855	95.97%	8962	8818	98.39%	5434	4869	89.60%
9948a_2ng	7686	8109	94.78%	6492	7814	83.08%	8798	9090	96.79%	7551	7600	99.36%
9948b_2ng	0	0		0	0		0	0		0	0	
9948c_2ng	8247	8649	95.35%	7739	7625	98.53%	8666	8960	96.72%	7147	7324	97.58%

Table 13. Genotypes of 9947A and 9948 DNA.

Locus	D3S1358 1	D3S1358 2	TH01 1	TH01 2	D5S818 1	D5S818 2	D13S317 1	D13S317 2	vWA 1	vWA 2	D8S1179 1	D8S1179 2
9947A	14	15	8	9.3	11	11	11	11	17	18	13	13
9948	15	17	6	9.3	11	13	11	11	17	17	12	13

Table 14. Peak height values of alleles detected in mixture samples. Mixture samples were prepared in the indicated 9947A to 9948 ratios. Peak height values (in RFU) are reported for all peaks observed at a locus. Highlighted values denote the exclusive minor component allele in each mixture.

Sample Info	D3S1358 (14)	D3S1358 (15)	D3S1358 (17)	TH01 (6)	TH01 (8)	TH01 (9.3)	D5S818 (11)	D5S818 (13)	D13S317 (11)	vWA (17)	vWA (18)	D8S1179 (12)	D8S1179 (13)
100: 0	1002	717			236	212	1449		1390	699	556		1152
95: 5	858	911	62		96		1509	128	1083	579	421	92	969
90: 10	1260	906	224	51	78	147	2016	273	2257	1152	796	239	1701
80: 20	572	767	190		92	87	1704	320	1189	970	461	276	1257
50: 50	522	1205	604	123	95	322	2140	724	2078	1158	267	504	1093
20: 80	198	1321	979	207		292	1077	834	1498	1237	139	737	1010
10: 90	157	918	844	154		237	1156	914	1389	1293	120	841	790
5: 95	139	1002	962	187		250	770	977	1593	1361		722	704
0: 100		1766	1823	309		383	1516	1221	2036	2106		1176	1168
9947	1151	1485			194	95	2012		1883	724	657		1845
9948		1789	1575	108		142	1170	1143	2466	1924		1321	1531
Negative													

Figure 1. BodePlex 3 allelic ladder.

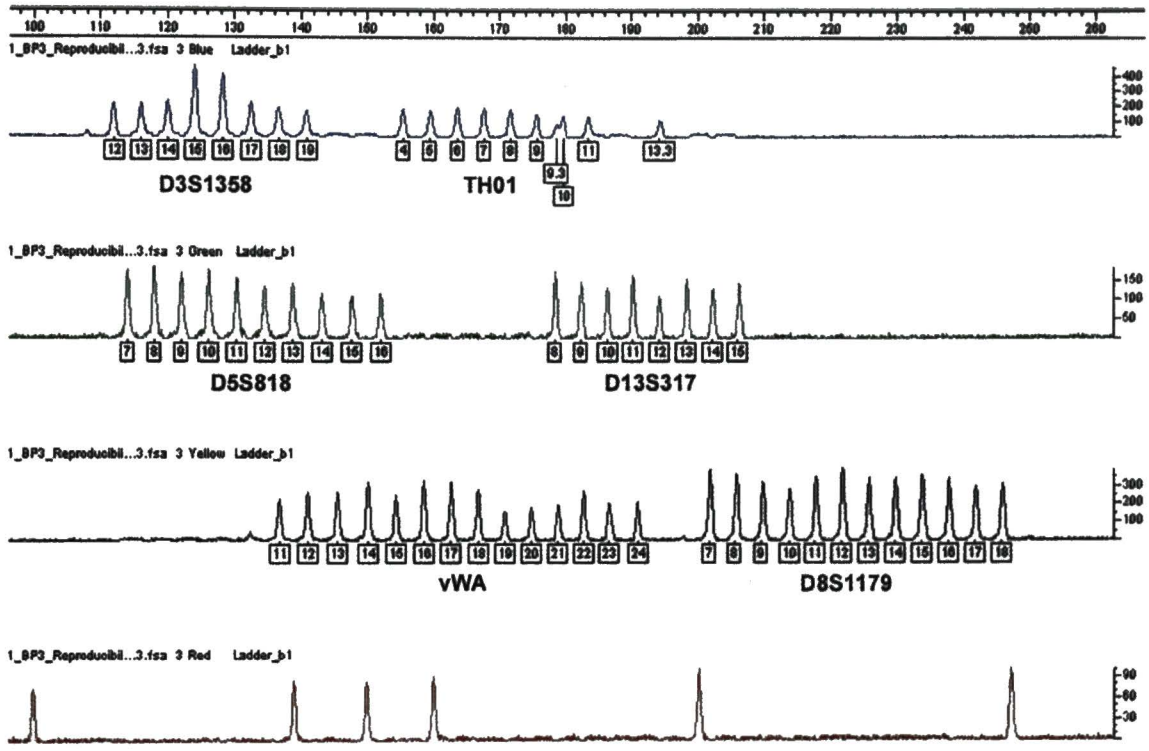


Figure 2. 9947A profile generated by BodePlex 3 multiplex. A 0.25 ng template of 9947A DNA was amplified with BodePlex 3 system.

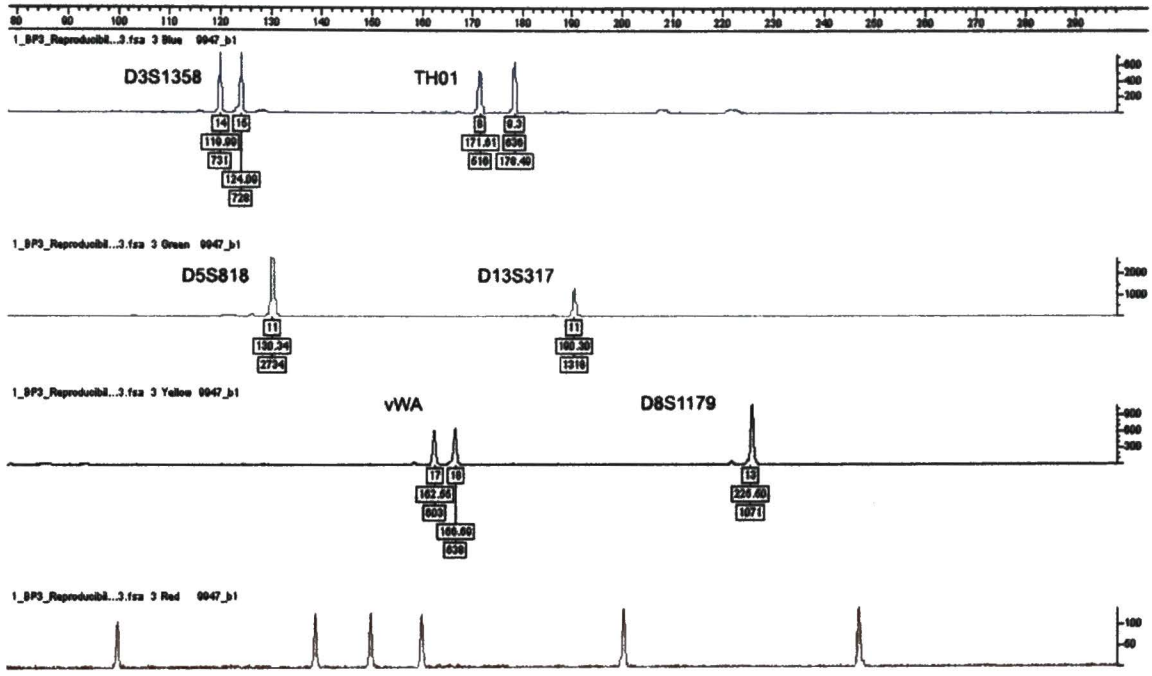
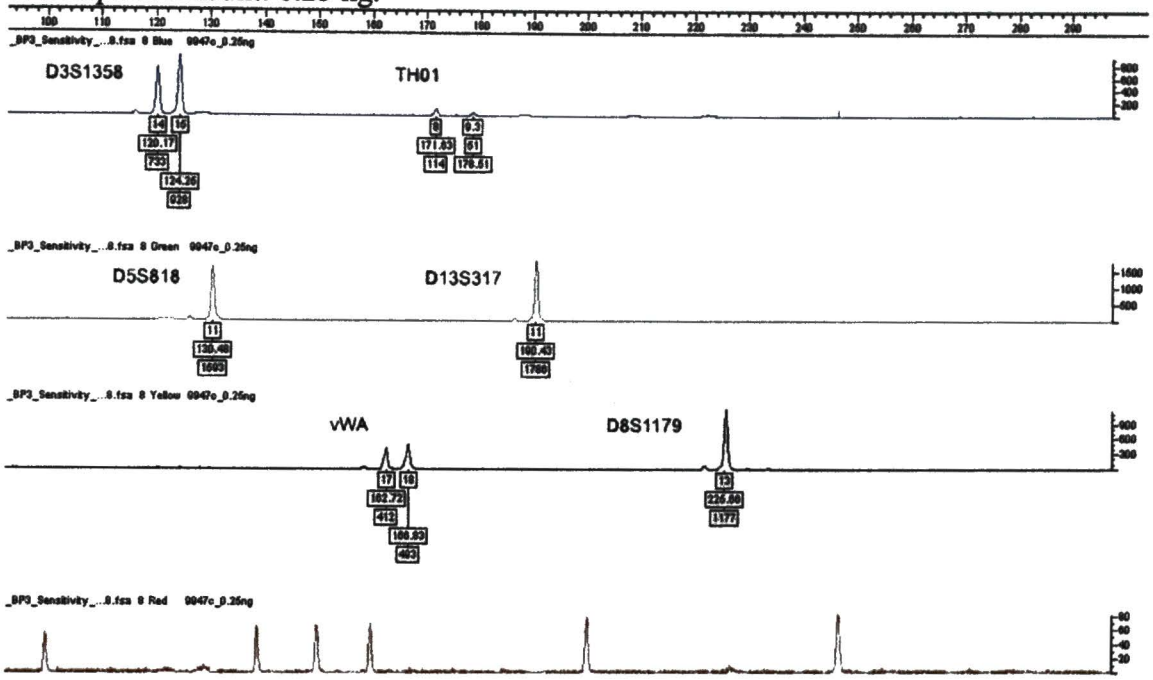


Figure 3. Profiles generated by 0.25 ng and 0.5 ng 9947A samples. Two different template amounts [0.25 ng (a) and 0.5 ng (b)] of 9947A were amplified with BodePlex 3.

a. Template amount: 0.25 ng.



b. Template amount: 0.5 ng.

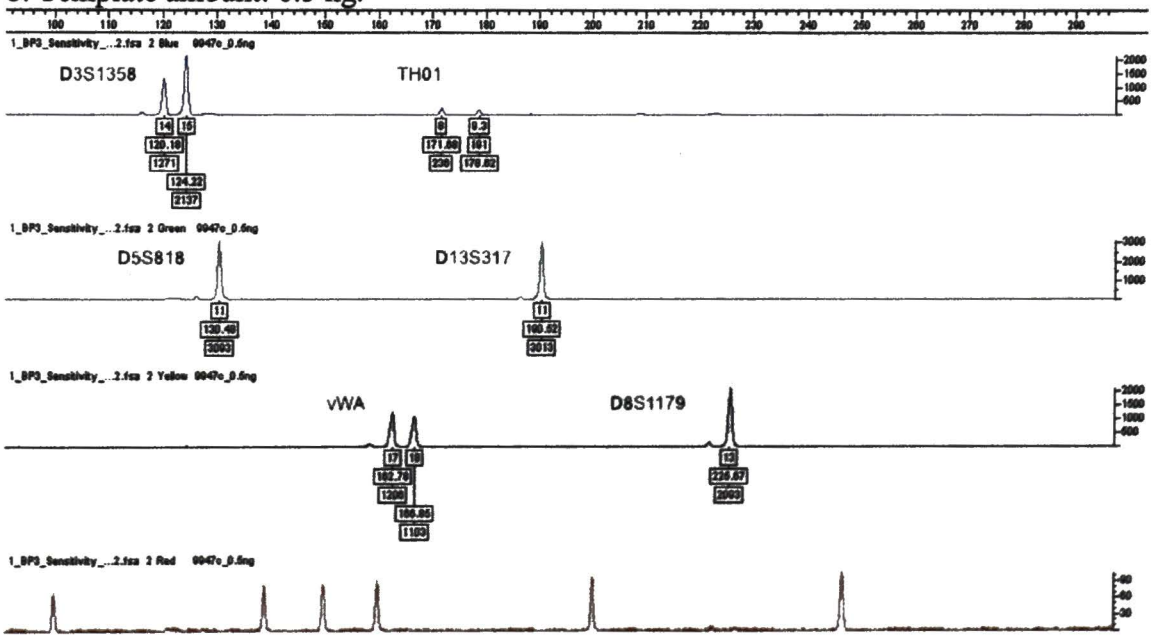
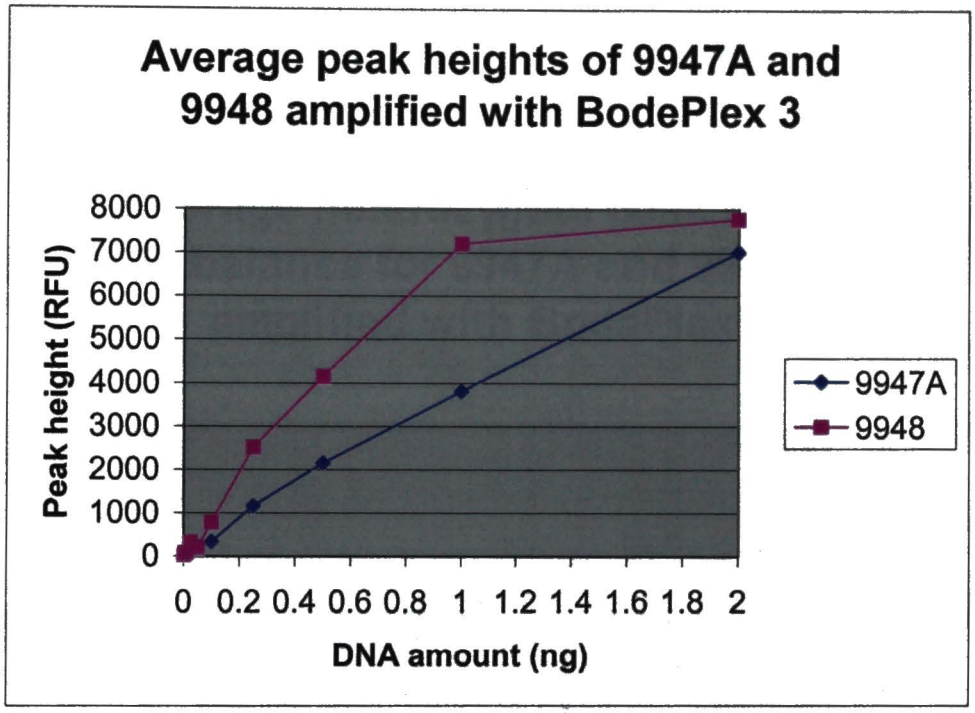


Figure 4. BodePlex 3 sensitivity - average peak heights of 9947A and 9948 samples amplified with BodePlex 3.



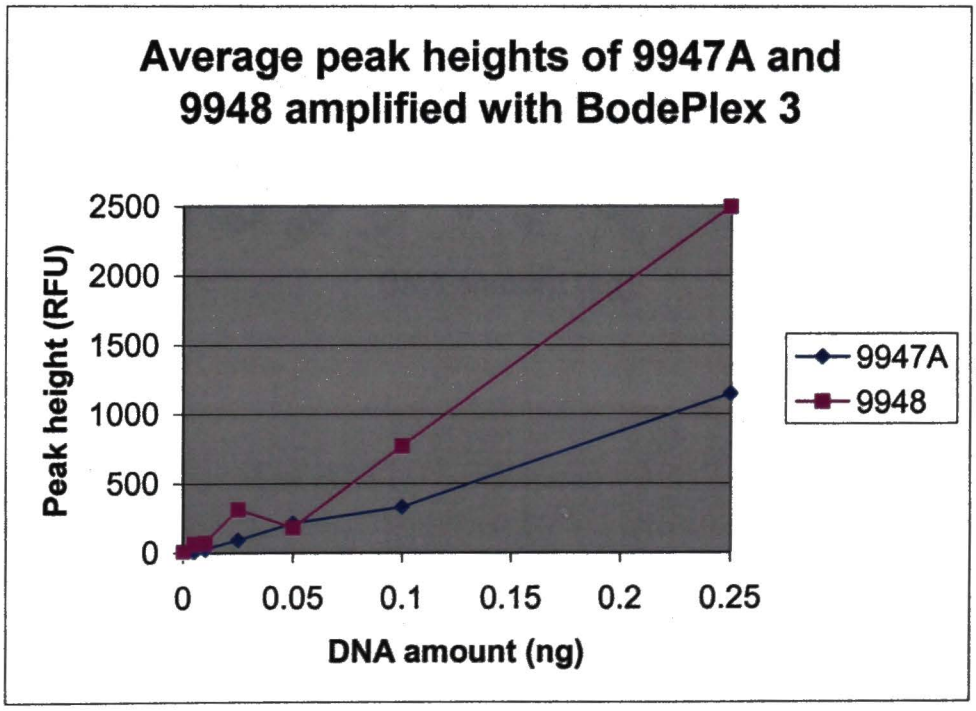


Figure 5. BodePlex 3 sensitivity - Average heterozygote peak height balance for 9947A and 9948 samples amplified with BodePlex 3. Percent of the average heterozygote peak height balance was calculated for all samples at different template amounts.

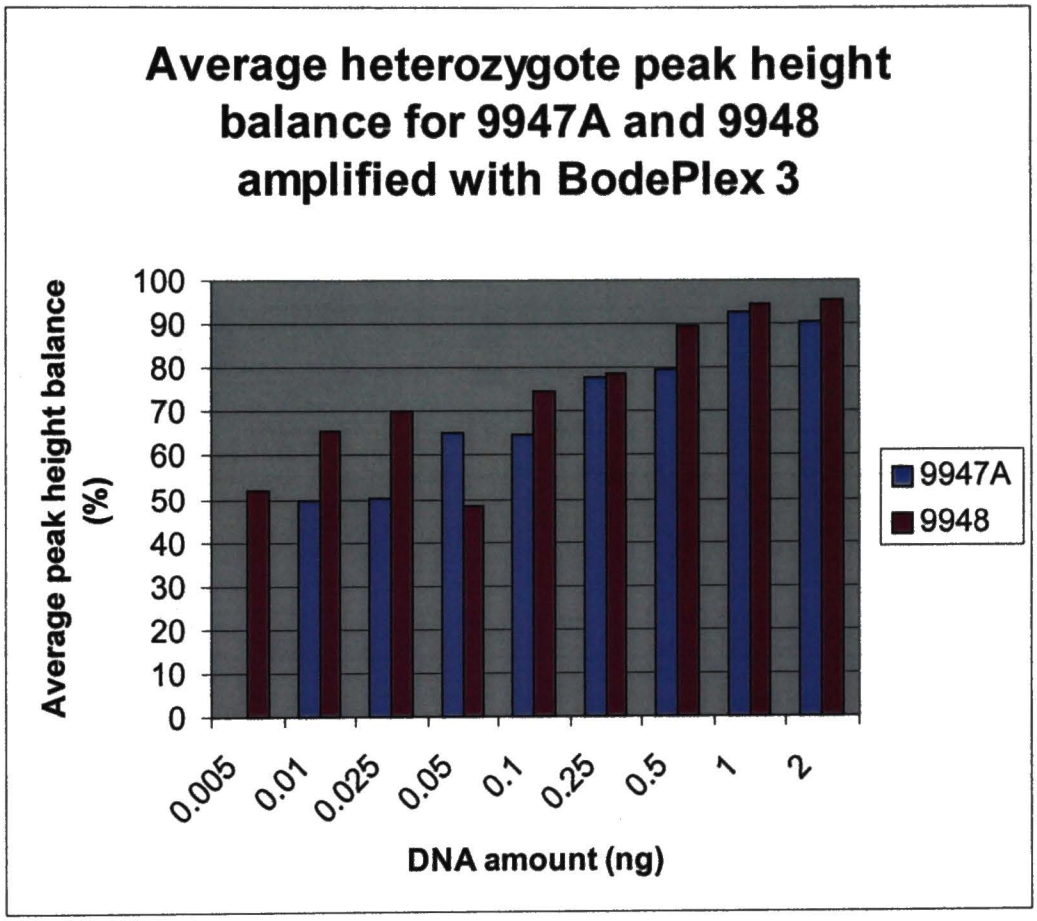
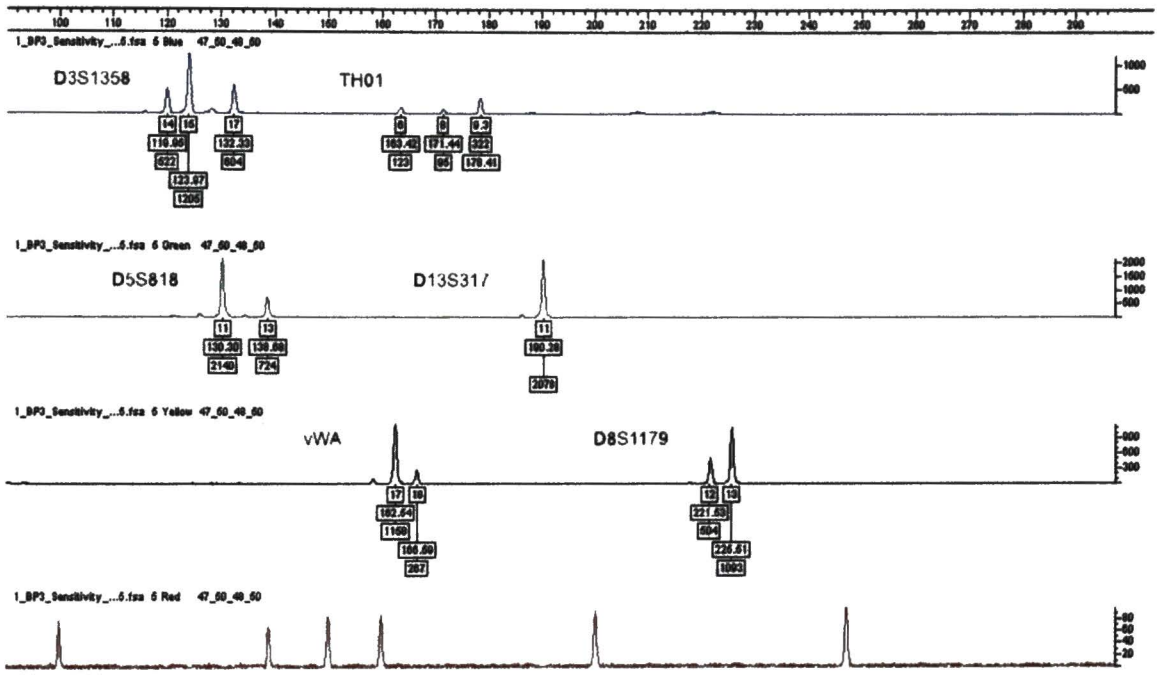


Figure 6. Profile of the 50:50 mixture sample amplified with BodePlex 3. The profile generated when a 50:50 mixture of 9947A to 9948 DNA was amplified with BodePlex 3 at a 0.25 ng template amount.



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