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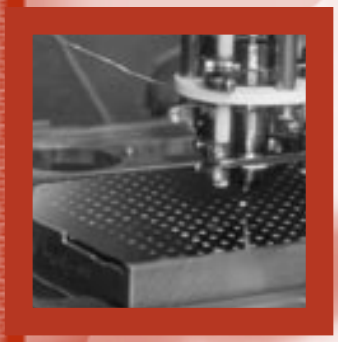

Improved Analysis of

# DNA Short Tandem Repeats

## With Time-of-Flight Mass Spectrometry

science and technology research report

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# Improved Analysis of DNA Short Tandem Repeats With Time-of-Flight Mass Spectrometry

*John M. Butler and Christopher H. Becker*

Science and Technology Research Report

October 2001
NCJ 188292

NIJ

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John M. Butler, Ph.D., is currently a research chemist at the National Institute of Standards and Technology and principle investigator on an NIJ-funded project to further develop multiplex PCR and time-of-flight mass spectrometry for future forensic DNA typing assays. He was the first to demonstrate that short tandem repeat typing could be performed with capillary electrophoresis.

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This project was supported under grant number 97–LB–VX–0003 from the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice.



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# ACKNOWLEDGMENTS

The project described in this report could not have happened without the hard work and support of a number of people at GeneTrace Systems, Inc. First and foremost, Jia Li did some of the early primer design and STR work to demonstrate that STRs could be effectively analyzed by mass spectrometry. Jia taught us a lot about PCR and was always encouraging of our work. Likewise, Tom Shaler was important in the early phases of this research with his expert advice in mass spectrometry and data processing. The first GeneTrace STR mass spectra were carefully collected by Tom, and thus he and Jia deserve credit for helping obtain the funding for this study. Dan Pollart synthesized numerous cleavable primers for this project, especially in the first year of our work. David Joo and Wendy Lam also prepared PCR and SNP primers for the later part of this work. A number of people assisted in robotic sample preparation and sample cleanup, including Mike Abbott, Jon Marlowe, David Wexler, and Rebecca Turincio. Joanna Hunter, Vera Delgado, and Can Nhan ran many of the STR samples on the automated mass spectrometers. Their hard work made it possible to focus on experimental design and data analysis rather than routine sample handling.

It was a great blessing to have talented and supportive coworkers throughout the course of this project. Kathy Stephens, Jia Li, Tom Shaler, Yuping Tan, Christine Loehrlein, Joanna Hunter, Hua Lin, Gordy Haupt, and Nathan Hunt provided useful discussions on a number of issues and helped develop assay parameters and tackle automation issues, among other things. Nathan Hunt was especially important to the success of this project because he developed the STR genotyping algorithm and CallSSR software as well as the multiplex SNP primer design software. Kevin Coopman developed the SNP genotyping algorithm and calling software and was always eager to analyze our multiplex SNP samples. Joe Monforte and Roger Walker served as our supervisors for the first year and second year of this project, respectively, which allowed us the opportunity to devote sufficient time to doing the work described in this project. Last but not least, Debbie Krantz served as an able administrator of these two NIJ grants and took care of the financial aspects.

We also were supported with samples and sequence information from a number of scientific collaborators. Steve Lee and John Tonkyn from the California Department of Justice DNA Laboratory provided genomic DNA samples and STR allelic ladders. Debang Liu from Northwestern University provided the D3S1358 DNA sequence used for improved primer design purposes. Peter Oefner and Peter Underhill from the Department of Genetics at Stanford University provided male population samples and Y-chromosome SNP sequences. The encouragement and support of Lisa Forman and Richard Rau from the Office of Justice Programs at the National Institute of Justice propelled this work from an idea to a working product. In addition, Dennis Reeder from the National Institute of Standards and Technology was always a constant source of encouragement at scientific meetings.

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List of exhibits included with this report:

# EXECUTIVE SUMMARY

## Introduction

The advent of DNA typing and its use for human identity testing has revolutionized law enforcement investigations in recent years by allowing forensic laboratories to match suspects with minuscule amounts of biological evidence from a crime scene. Equally important is the use of DNA to exclude suspects who were not involved in a crime or to identify human remains in an accident.

The past decade has seen numerous advances in the DNA testing procedures, most notably among them the development of PCR (polymerase chain reaction)-based DNA typing methods. Technologies for measuring DNA variations, both length and sequence polymorphisms, have also advanced rapidly in the past decade. The time needed to determine a sample's DNA profile has dropped from 6–8 weeks to 1–2 days, and with more recent advancements, the time needed to process samples may decrease to as little as a few hours, maybe even a few minutes.

Simultaneous with the evolution of DNA markers and technologies embraced by the forensic community has been the acceptance and use of DNA typing information. The courtroom battles over statistical issues that were common in the late 1980s and early 1990s have subsided as DNA evidence has become more widely accepted.

In the past 5 years, DNA databases have emerged as powerful tools for criminal investigations, much like the fingerprint databases that have been used routinely for decades.

The United Kingdom launched a nationwide DNA database in 1995 that now contains more than 1 million DNA profiles of convicted felons—profiles that have been used to aid more than 75,000 criminal investigations. National DNA databases are springing up in countries all over the world as their value to law enforcement is being recognized.

In the United States, the FBI has developed the Combined DNA Index System (CODIS) with the anticipation that several million DNA profiles will be entered into this database in the next decade. All 50 States now have laws requiring DNA typing of convicted offenders, typically for violent crimes such as rape or homicide.

While the law enforcement community is gearing up to gather millions of DNA samples from convicted felons, the DNA typing technology needs improvement. Large backlogs of samples exist today due to the high cost of performing the DNA testing and limited capabilities in forensic laboratories. As of the summer of 1999, several States, including California, Virginia, and Florida, had backlogs of more than 50,000 samples. A need exists for more rapid and cost-effective methods for high-throughput DNA analysis to process samples currently being gathered for large criminal DNA databases around the world.

At the start of this project in June 1997, commercially available slab gel or capillary electrophoresis instruments could handle only a few dozen samples per day. While larger numbers of samples can be processed by increasing the number of laboratory personnel and instruments, the development of high-throughput DNA processing technologies promises to be more cost effective in the long run, especially for the generation of large DNA databases. GeneTrace Systems, Inc., a small biotechnology company located in Alameda, California, has developed high-throughput DNA analysis capabilities using time-of-flight mass spectrometry coupled with parallel sample preparation on a robotic workstation. The GeneTrace technology allows several thousand samples to be processed daily. DNA samples can be analyzed in seconds, rather than minutes or hours, and with improved accuracy compared with conventional electrophoresis methods.

Overall, the mass spectrometry method described in this study is two orders of magnitude faster in sample processing time than conventional techniques.

## Purpose of the Report

This NIJ project was initiated to adapt the GeneTrace technology to human identity DNA markers commonly used by forensic DNA laboratories, specifically short tandem repeat (STR) markers. An extension of the original grant was submitted in December 1997 to fund the development of single nucleotide polymorphism (SNP) markers from mitochondrial DNA and the Y chromosome.

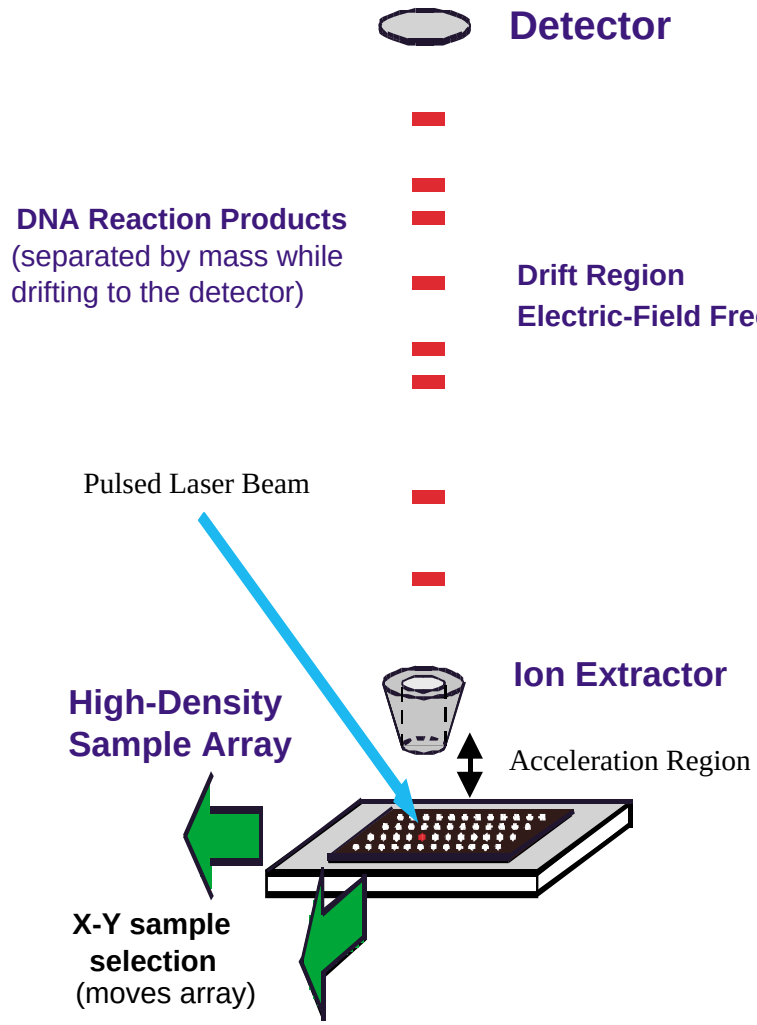
Based on the results obtained in this study, the authors believe mass spectrometry can be a useful and effective means for high-throughput DNA analysis, and that it has the capabilities to meet the needs of the forensic DNA community for offender DNA databases.

However, due to limited resources and a perceived difficulty to enter the forensic DNA market, GeneTrace made a business decision to not pursue this market. While the STR milestones on the original grant were met, only the initial milestones were achieved on the SNP portion of the NIJ grant because of the premature termination on the part of GeneTrace.

GeneTrace Systems, Inc., developed an integrated high-throughput DNA analysis system involving the use of proprietary chemistry, robotic sample manipulation, and time-of-flight mass spectrometry. The purpose of this NIJ project was to apply the GeneTrace technology to improve the analysis of STR markers commonly used in forensic DNA laboratories.

Mass spectrometry is a versatile analytical technique that involves the detection of ions and the measurement of their mass-to-charge ratio. Because these ions are separated in a vacuum environment, analysis times can be extremely rapid, often within microseconds. Many advances have been made in the past decade for the analysis of biomolecules such as DNA, proteins, and carbohydrates since the introduction of a new ionization technique known as matrix-assisted laser desorption-ionization (MALDI) and the discovery of new matrixes that effectively ionize DNA without extensive fragmentation. When coupled with time-of-flight mass spectrometry, this method for measuring biomolecules is commonly referred to as MALDI-TOF-MS. A schematic of MALDI-TOF-MS is presented in exhibit 1.

Exhibit 1. **Schematic of GeneTrace automated time-of-flight mass spectrometer.** DNA molecules are liberated from a solid-phase matrix environment with a laser pulse. The DNA reaction products are separated by size (mass) in a matter of microseconds, as opposed to hours using conventional methods. For each run, hundreds of samples are prepared in parallel using a robotic workstation and spotted on a sample plate that is introduced to the vacuum environment of the mass spectrometer. The sample plate moves under the fixed laser beam to allow sequential sample analysis.




## Short Tandem Repeats

Short tandem repeat (STR) DNA markers, also referred to as microsatellites or simple sequence repeats (SSRs), consist of tandemly repeated DNA sequences with a core repeat of 2–6 base pairs (bp). STR markers are readily amplified during PCR by using primers that bind in conserved regions of the genome flanking the repeat region. Forensic laboratories prefer tetranucleotide loci (i.e., 4 bp in the repeat) due to the lower amount of "stutter" produced during PCR. (Stutter products are additional peaks that can complicate the interpretation of DNA mixtures by appearing in front of regular allele peaks.) The number of repeats can vary from 3 or 4 repeats to more than 50 repeats with extremely polymorphic markers. The number of repeats, and hence the size of the PCR product, may vary among samples in a population making STR markers useful in identity testing or genetic mapping studies.

Shortly after this project was initiated, the FBI designated 13 core STR loci for the nationwide CODIS database. These STR loci are TH01, TPOX, CSF1PO, VWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11. The sex-typing marker, amelogenin, is also included in STR multiplexes that cover the 13 core STR loci. Each sample must have these 14 markers tested to be entered into CODIS.

To illustrate the kinds of numbers involved to analyze the current national sample backlog of ~500,000 samples, more than 7 million genotypes must be generated. Using currently available technologies, an estimated $25 million (~$50/sample) and more than 5 years for well-trained and well-funded

laboratories would be required to determine those 7 million genotypes. With the high cost and effort required, most of these backlogged samples are being stored in anticipation of future analysis and inclusion in CODIS, pending the development of new, faster technology or the implementation of more instruments using the current electrophoresis technologies.

Exhibit 2. **PCR product sizes with newly designed primers for commonly used STR loci compared with commercially available primers used in multiplex sets for fluorescence-based assays.** The red numbers indicate PCR product size ranges that exceed the recommended 140 bp mass spectrometry detection range.

| STR Locus | Known Alleles | GeneTrace Sizes *(newly designed primers in this study)* | Commercially Available Sizes* | |
|---|---|---|---|---|
| | | | Applied Biosystems | Promega |
| **Amelogenin** | X, Y | 106, 112 bp | 106, 112 bp | 212, 218 bp |
| **CD4** | 4–15 | 81–136 bp | N.A. | |
| **CSF1PO** | 6–15 | 87–123 bp | 280–316 bp | 291–327 bp |
| **F13A1** | 3–17 | 112–168 bp | N.A. | 279–335 bp |
| **F13B** | 6–12 | 110–134 bp | N.A. | 169–193 bp |
| **FES/FPS** | 7–15 | 76–108 bp | N.A. | 222–254 bp |
| **FGA** | 15–30 | 118–180 bp | 206–266 bp | N.A. |
| **D3S1358** | 9–20 | 76–120 bp | 101–145 bp | N.A. |
| **D5S818** | 7–15 | 89–121 bp | 134–166 bp | 119–151 bp |
| **D7S820** | 6–14 | 66–98 bp | 257–289 bp | 215–247 bp |
| **D8S1179** | 8–18 | 92–130 bp | 127–167 bp | N.A. |
| **D13S317** | 7–15 | 98–130 bp | 201–233 bp | 165–197 bp |
| **D16S539** | 5,8–15 | 81–121 bp | 233–273 bp | 264–304 bp |
| **D18S51** | 9–27 | 120–192 bp | 272–344 bp | N.A. |
| **D21S11** | 24–38 | 150–190 bp | 186–242 bp | N.A. |
| **DYS19** | 8–16 | 76–108 bp | N.A. | |
| **DYS391** | 9–12 | 99–111 bp | N.A. | |
| **HPRTB** | 6–17 | 84–128 bp | N.A. | 259–303 bp |
| **LPL** | 7–14 | 105–133 bp | N.A. | 105–133 bp |
| **TH01** | 3–13.3 | 55–98 bp | 160–203 bp | 171–214 bp |
| **TPOX** | 6–14 | 69–101 bp | 217–249 bp | 224–256 bp |
| **VWA** | 11–22 | 126–170 bp | 156–200 bp | 127–171 bp |
| ***Other STRs*** | | | | |
| GATA132B04 | 10–14 | 99–115 bp | N.A. | |
| D22S445 | 10–16 | 110–130 bp | N.A. | |
| D16S2622 | 4–8 | 71–87 bp | N.A. | |

* Sizes are listed without adenylation (add 1 base for +A form).
N.A. = Not Available.

Time-of-flight mass spectrometry has the potential to bring DNA sample processing to a new level in terms of high-throughput analysis. However, there are several challenges to using MALDI-TOF-MS for the analysis of PCR products, such as STR markers. Mass spectrometry resolution and sensitivity are diminished when either the DNA size or the salt content of the sample is too large. By redesigning the PCR primers to bind close to the repeat region, the STR allele sizes are reduced to benefit the resolution and sensitivity of the PCR products. Therefore, much of this project involved designing and testing new PCR primers that produced smaller amplicon sizes for STR markers of forensic interest. This research focused on STR loci that have been developed by commercial manufacturers and studied extensively by forensic scientists. These include all of the GenePrint™ tetranucleotide STR systems from Promega Corporation (Madison, WI) as well as the 13 CODIS STR loci that are covered by the Profiler Plus™ and COfiler™ kits from Applied Biosystems (ABI) (Foster City, CA) (exhibit 2). Where possible, primers were designed to produce amplicons less than 100 bp in size, although it has been possible to resolve neighboring STR alleles as large as 140 bp. For example, TPOX alleles 6–14 ranged from 69–101 bp in size with GeneTrace-designed primers; while with Promega's GenePrint™ primers, the same TPOX alleles ranged in size from 224–256 bp. Unfortunately, due to the long and complex repeat structures of several STR markers, this study was unable to obtain the necessary single-base resolution with the following STR loci: D21S11, D18S51, and FGA (see Results and Discussion of STR Analysis by Mass Spectrometry).

To verify the STR results obtained from the mass spectrometry method, the authors collaborated with the California Department of Justice (CDOJ) DNA Laboratory in Berkeley to generate a large data set. CDOJ provided 88 samples that had been previously genotyped

using validated fluorescent multiplex STR kits from ABI. GeneTrace generated STR results using their primer sets for 9 STR loci (TH01, TPOX, CSF1PO, D3S1358, D16S539, D8S1179, FGA, DYS391, and D7S820) along with the sex-typing marker amelogenin. These experiments compared more than 700 genotypes (88 samples × 8 loci; data from D8S1179 and DYS391 were not available from CDOJ). Although results were not obtained for all possible samples using mass spectrometry, researchers observed almost 100% correlation with the genotypes obtained between the validated fluorescent STR method and GeneTrace's newly developed mass spectrometry technique, demonstrating that the GeneTrace method was reliable (see Results and Discussion of STR Analysis by Mass Spectrometry).

## Multiplex STR analysis

To reduce analysis cost and sample consumption and to meet the demands of higher sample throughputs, PCR amplification and detection of multiple markers (multiplex STR analysis) has become a standard technique in most forensic DNA laboratories. STR multiplexing is most commonly performed using spectrally distinguishable fluorescent tags and/or nonoverlapping PCR product sizes. An example of an STR multiplex produced from a commercially available kit is shown in exhibit 3. Multiplex STR amplification in one or two PCR reactions with fluorescently labeled primers and measurement with gel or capillary electrophoresis separation and laser-induced fluorescence detection is becoming a standard method among forensic laboratories for analysis of the 13 CODIS STR loci. The STR alleles from these multiplexed PCR products typically range in size from 100–350 bp with commercially available kits.

Due to the limited DNA size constraints of mass spectrometry, GeneTrace adopted a different approach to multiplex analysis of multiple STR loci. Primers

Exhibit 3. **Fluorescent multiplex STR result with AmpFlSTR® COfiler™ kit and ABI 310 Genetic Analyzer.** The red peaks are an internal sizing standard (GS350-ROX). The DNA size window shown here is 90–350 bp. The allele calls for the seven loci in this multiplex are amelogenin: X,Y; D3S1358: 15, 17; TH01: 6, 7; TPOX: 8, 12; D16S539: 8, 12; D7S820: 9, 11; and CSF1PO: 12, 12.

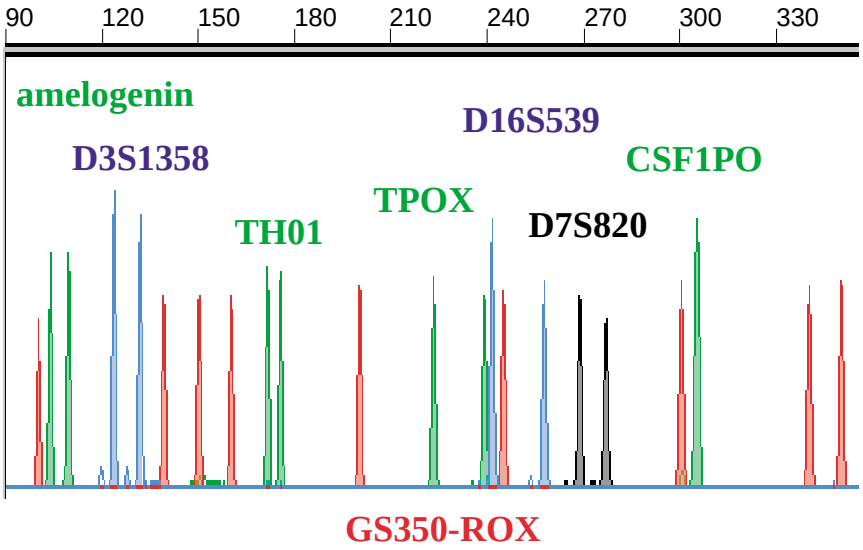



Exhibit 4. **Schematic of expected allele masses for a CSF1PO-TPOX-TH01 (CTT) multiplex involving overlapping allele size ranges.** All known alleles are fully distinguishable by mass with this interleaving approach.

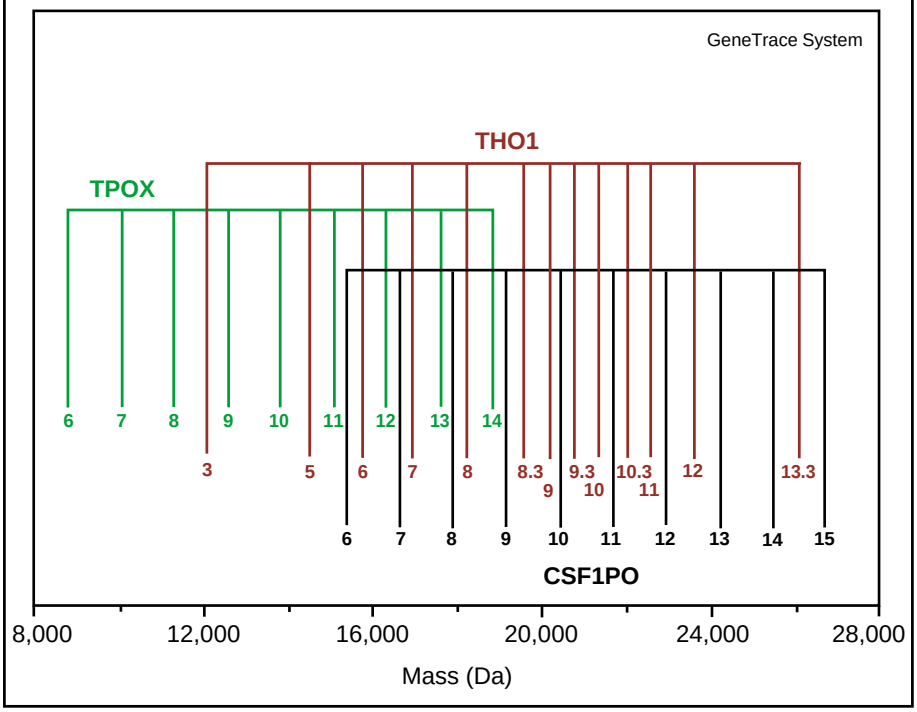
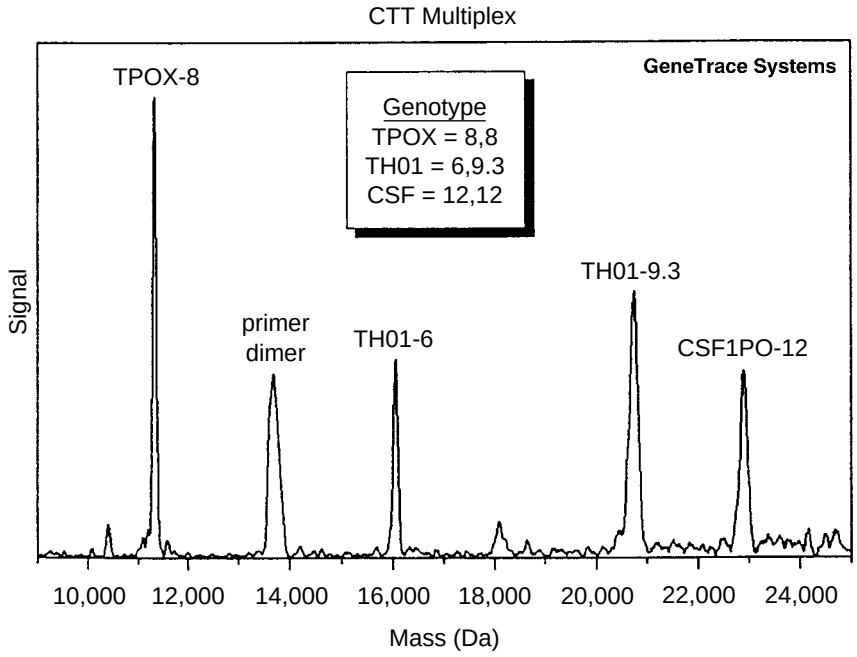


Exhibit 5. **Mass spectrum of an STR triplex involving TPOX, TH01, and CSF1PO.** This sample is a mass spectrometry result using the interleaving allele approach schematically illustrated in exhibit 4. Multiplex PCR and multiplex primer extension with ddC termination were used to obtain this result.




are designed such that the PCR product size ranges overlap between multiple loci but have alleles that interleave and are resolvable in the mass spectrometer (exhibit 4). As described above, PCR primers are closer to the STR repeat regions than those commonly used with electrophoresis systems. The high accuracy, precision, and resolution of this mass spectrometry approach permits multiplexing STR loci for a limited number of markers. During the study, GeneTrace also developed a TH01-TPOX-CSF1PO STR triplex (exhibit 5).

## Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) represent another form of DNA variation that is useful for human identity testing. SNPs are the most frequent form of DNA sequence variation in the human genome and are becoming increasingly popular genetic markers for genome mapping studies and medical diagnostics. SNPs are typically biallelic with two possible nucleotides (nt) or alleles at a particular site in the genome. Because SNPs are less polymorphic (i.e., have fewer alleles) than the currently used STR markers, more SNP markers are required to obtain the same level of discrimination between samples. Current estimates are that 30–50 unlinked SNPs will be required to obtain the matching probabilities of 1 in ~100 billion as seen with the 13 CODIS STRs.

The perceived value of SNPs for DNA typing in a forensic setting include the following:

- More rapid analysis.
- Cheaper costs.
- Simpler interpretation of results because there are no stutter products.
- Improved ability to handle degraded DNA because of the possibility of smaller PCR product sizes.

While it is doubtful that autosomal SNPs will replace the current battery of STRs used in forensic laboratories in the near future, abundant mitochondrial and Y-chromosome SNP markers exist and have already proven useful as screening tools. These maternal (mitochondrial) and paternal (Y chromosome) lineage markers are effective in identifying missing persons and war casualties and helping answer historical questions such as whether or not Thomas Jefferson fathered a slave child.

The forensic DNA community already has experience with applying SNP markers as a screening process, which can prove very helpful for excluding suspects from crime scenes. Many crime laboratories still use reverse dot blot technology for analyzing the SNPs from HLA-DQA1 and PolyMarker loci with kits from ABI. In addition, mitochondrial DNA (mtDNA) sequencing is currently performed in some forensic laboratories.

In the work performed on multiplex SNP markers at GeneTrace, the authors examined 10 polymorphic sites within the mtDNA control region and 20 Y-chromosome SNPs provided by Dr. Peter Oefner and Dr. Peter Underhill from Stanford University. A multiplex SNP assay was developed for 10 mtDNA SNP sites (exhibit 6). Only limited work was performed on the Y-chromosome SNPs due to the premature termination of the work. However, results demonstrated a male-specific 17-plex PCR of 17 different Y SNP markers (exhibit 7).

## Conclusions and Implications

Time-of-flight mass spectrometry offers a rapid, cost-effective alternative for genotyping large numbers of samples. Each DNA sample can be accurately measured in a few seconds. Due to the increased accuracy of mass spectrometry, STR alleles can be reliably typed without comparison with allelic ladders. Mass spectrometry holds significant promise as a technology for high-throughput DNA processing that will be valuable for large-scale DNA database work.

In summary, the positive features of mass spectrometry for STR analysis include:

- Rapid results—STR typing at a rate of seconds per sample.
- Accuracy—no allelic ladders.
- Direct DNA measurement—no fluorescent or radioactive labels.
- Automated sample preparation and data collection.
- High-throughput capabilities of thousands of samples daily per system.
- Flexibility—single nucleotide polymorphism (SNP) assays can be run on the same instrument platform.

This project demonstrates that both STR and SNP analysis are reliably performed with GeneTrace's mass spectrometry technology. Tests were done on a large number of human DNA markers of forensic interest. New primer sets were developed for the 13 CODIS STR loci that may prove useful in the future for situations in which degraded DNA is present and requires smaller amplicons to obtain successful results. The possibility of developing multiplexed SNP markers also was explored, and a mtDNA 10-plex assay and Y-chromosome, 17-plex, male-specific PCR were demonstrated. Both STR and SNP areas appear promising for future research. In another project, GeneTrace recently demonstrated a sample throughput of approximately 4,000 STR samples in a single day with a single automated mass spectrometer. Clearly, this is an improvement in the analysis of DNA short tandem repeat markers using time-of-flight mass spectrometry.

Exhibit 6. **Mass spectrum of SNP 10-plex assay for screening polymorphic sites in the mtDNA control region.** The bottom panel shows the 10 SNP primers prior to the primer extension reaction. The top panel contains the multiplexed reaction products, each labeled with the observed extension product. The results for this K562 PCR product are (in order across HV1 and HV2): H16069 (G), H16129 (C), H16189 (A), H16224 (G), H16311 (A), H00073 (C), L00146 (T), H00152 (A), L00195 (T), and H00247 (C). SNP nucleotide results have been confirmed by sequencing. The primer sequences are listed in exhibit 24. The primer concentrations were 25 pmol MT5, 15 pmol MT8', 15 pmol MT10, 10 pmol MT3', 20 pmol MT9, 25 pmol MT6, 20 pmol MT2g, 27 pmol MT1, 35 pmol MT7, and 20 pmol MT4e.

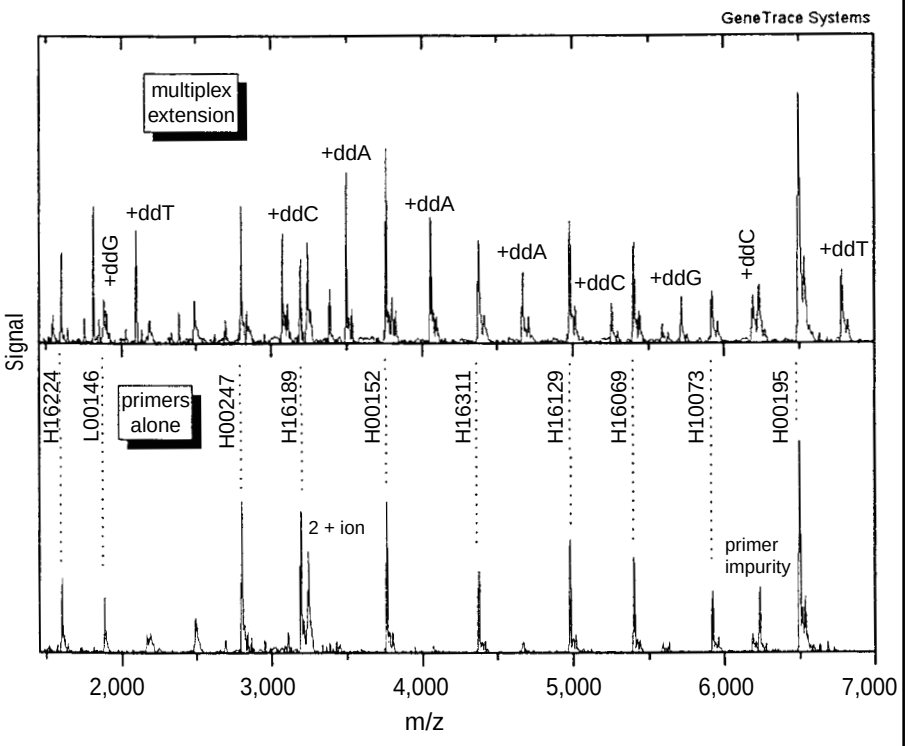
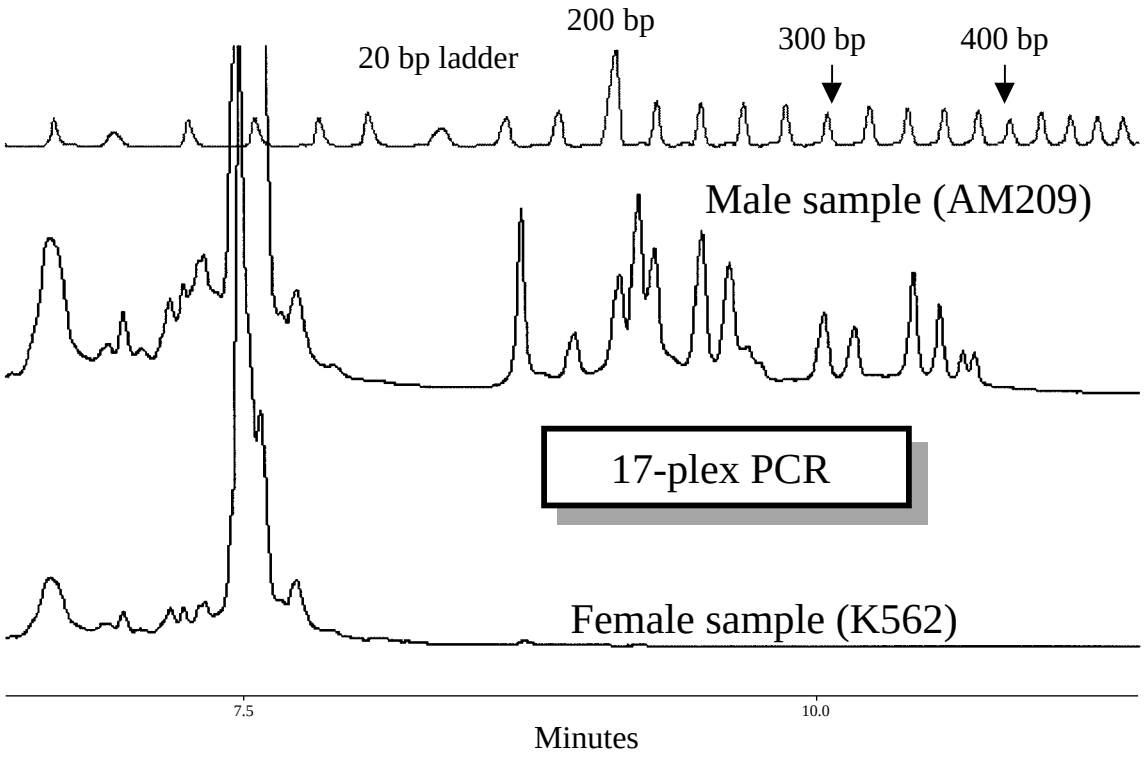


Exhibit 7. **Overlay of CE electropherograms demonstrating male-specific amplification with the 17-plex set of PCR primers.** Note: The female sample (K562) failed to yield any peaks, illustrating that the PCR reaction is Y chromosome-specific. The PCR primers used are described in exhibit 27 with each locus-specific primer set at 0.4 pmole each. For the PCR reaction conditions, see exhibit 67 and details in the text. The 17 amplicons may be seen more clearly in exhibit 68.



## STR Grant

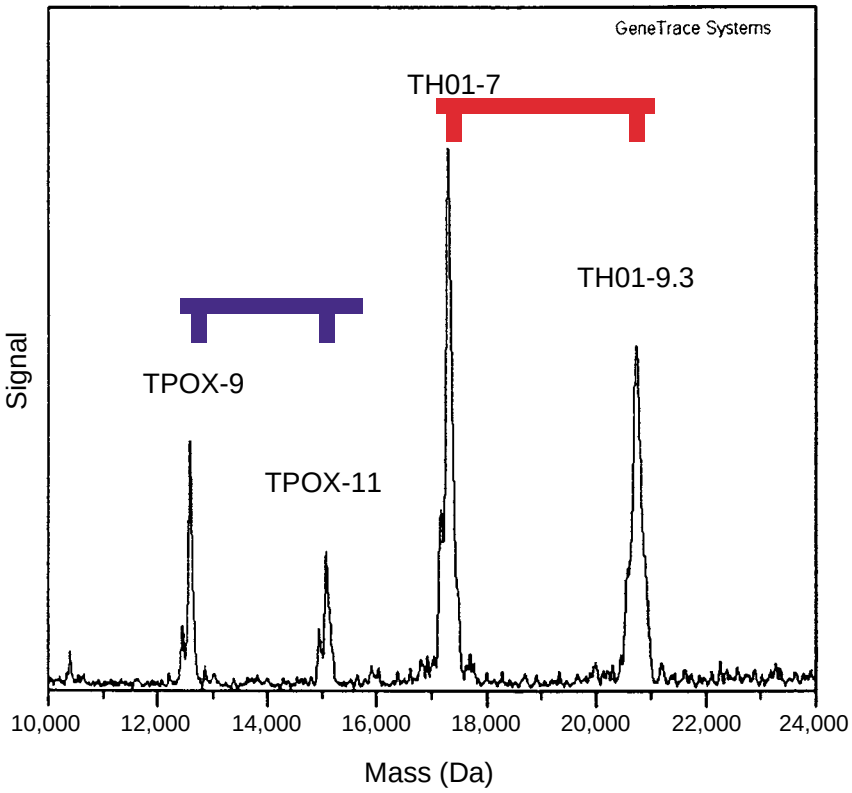
This project focused on the development of a powerful new technology for rapid and accurate analysis of DNA STR markers using time-of-flight mass spectrometry. GeneTrace Systems, Inc., collaborated with the CDOJ DNA Laboratory in Berkeley, California, primarily through Dr. Steve Lee. This collaboration provided the study with the samples used to verify the new GeneTrace technology, which was done by comparing the mass spectrometry results with genotypes obtained using established and validated methods run at CDOJ.

To accomplish the task of developing a new mass spectrometry technology for STR typing, five milestones were proposed in the original grant application, which included the following:

- Redesign PCR primers for a number of commonly used STR markers to produce smaller PCR products that could be tested in the mass spectrometer (exhibit 2).
- Demonstrate multiplexing capabilities to a level of 2 or 3 for detection with the TOF-MS method (exhibits 4–5 and 8–10).
- Transfer the sample preparation protocols from manual to a highly parallel and automated pipetting robot.
- Develop a large data set to confirm the accuracy and reliability of this method (exhibits 11–19).
- Automate and incorporate DNA extraction techniques onto the GeneTrace robots.

As described in the results section, all milestones were met on time except the final one regarding DNA extraction. Two other companies, Rosys and Qiagen, produced robotic systems for DNA extraction after this project began. Meanwhile, GeneTrace remained focused on developing other steps in DNA sample processing since commercially available solutions had already been developed, thereby eliminating the need to include the DNA extraction portion in this study.

Exhibit 8. **Mass spectrum of an STR multiplex sample with nonoverlapping alleles.** The two loci, TH01 and TPOX, were coamplified using the ddC termination approach. Note: The mass accuracy is improved for peaks closer to the calibration standard of 10,998 Da.




| | Expected Mass | Observed Mass | Δ Mass |
|---|---|---|---|
| TPOX-9 | = 12,581 Da | 12,584 Da | +3 Da |
| TPOX-11 | = 15,101 Da | 15,080 Da | -21 Da |
| TH01-7 | = 17,336 Da | 17,305 Da | -31 Da |
| TH01-9.3 | = 20,803 Da | 20,751 Da | -52 Da |

Since this project began in June 1997, a number of advances that impact the ability to perform high-throughput DNA typing have occurred in the biotech field. In early 1998, ABI released a dual 384-well PE9700 ("Viper") thermal cycler, which makes it possible to prepare 768 PCR samples simultaneously. Beckman Instruments also came to market with a 96-tip Multimek pipetting robot. At the beginning of this project, GeneTrace used funds from this NIJ grant to purchase an MJ Research 384-well thermal cycler and a custom-built 96-tip robotic pipettor on a CyberLab x-y-z gantry. Both of these pieces of equipment were the state of the art at the time but are now obsolete at GeneTrace for routine operations and have been replaced by the newer and more reliable products from Applied Biosystems and Beckman Instruments.

Exhibit 9. **Mass spectrum of STR multiplex mixture of TH01 and TPOX allelic ladders.** The PCR products from the two loci differ by 120 Da. The ladders were reamplified from AmpF1STR® Green I kit materials. The TH01 ladder ranges from 5 to 10 repeats with 9.3 included; the TPOX ladder ranges from 6 to 13 repeats with 13 not shown here.

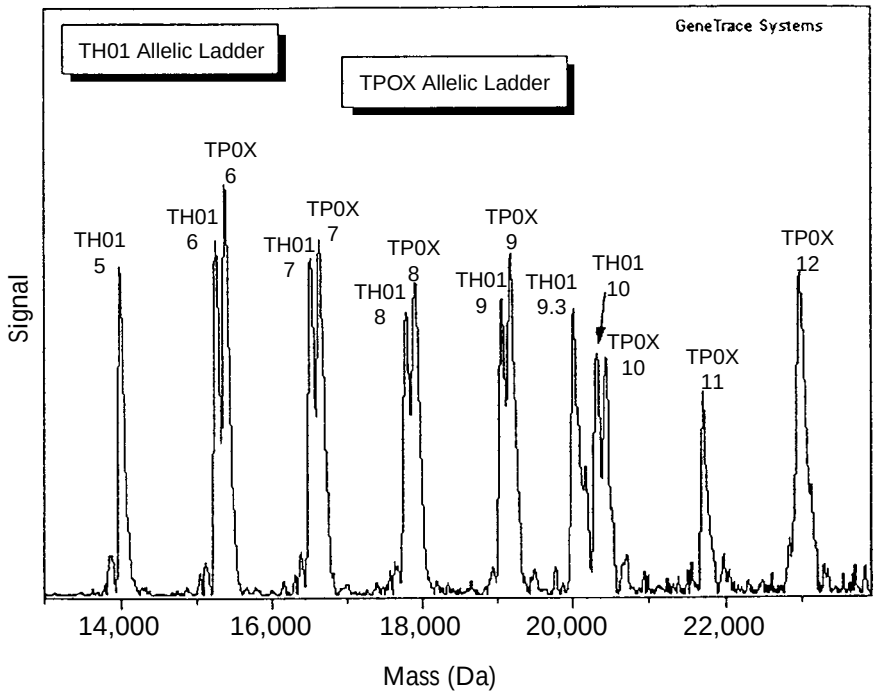
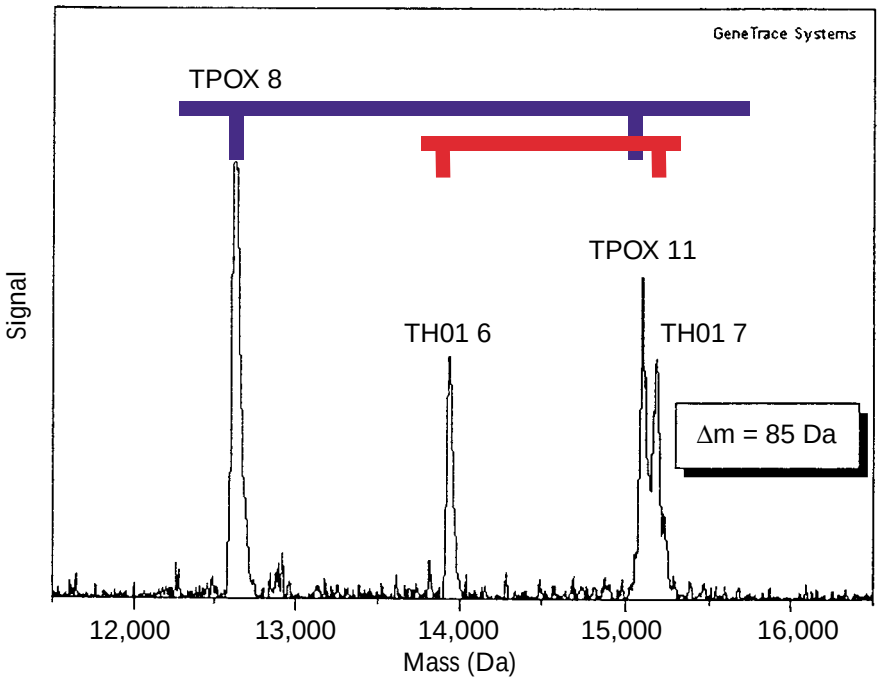



Exhibit 10. **Mass spectrum of a multiplexed TH01 and TPOX sample showing interleaved amplicons that are only 85 Da apart**




## SNP Grant

The grant extension, which began in August 1998, focused on the development of multiplexed SNP markers from mtDNA and the Y chromosome. Although the grant extension was terminated prematurely by GeneTrace management in April 1999, portions of the first four milestones were accomplished. The five milestones described in the original grant extension included the following:

- Produce and test a set of 10 or more SNP probes for mtDNA control region "hot spots" (exhibit 5).
- Develop software for multiplex SNP analysis and data interpretation.
- Examine individual Y-chromosome SNP markers.
- Develop multiplex PCR and multiplex SNP probes for Y-chromosome SNP loci (exhibit 7).
- Determine the discriminatory power for a set of Y-chromosome markers by running ~300 samples across 50 Y-chromosome SNP markers.

The goal of the grant extension project was to develop highly multiplexed SNP assays that worked in a robust manner with mass spectrometry and could be genotyped in an automated fashion. GeneTrace planned to select markers with a high degree of discrimination to aid in rapid screening of mitochondrial DNA and Y-chromosome polymorphisms with the capability to handle analysis of large databases of offender DNA. At the time this proposal was written (December 1997), GeneTrace still intended to provide reagents and instruments to large DNA service laboratories or to provide a DNA typing service to the forensic DNA community. The grant extension was prematurely terminated due to a change in business focus and a need to consolidate the research efforts at GeneTrace.

Exhibit 11. **STR data collection times for CDOJ samples.** Comparing ABI 310 run times with multiple mass spectrometry runs indicates that data collection speed increased almost 10-fold during 1998 due to a number of improvements in the GeneTrace process. Data collection speed is a combination of laser rate, total number of shots taken, sample cleanliness, and other factors. Overall, the mass spectrometry data collection speed is approximately two orders of magnitude faster than the ABI 310. Exhibits 12–19, 39, and 40 list the observed masses for these samples measured by GeneTrace's mass spectrometry method.

**ABI 310 Runs**

January 5, 1999–January 8, 1999:

- Multiplex STR detection and analysis (COfiler™ kit amplifies 6 STRs + amelogenin simultaneously: TH01, TPOX, CSF1PO, D3S1358, D16S539, D7S820)
- ~54 hours of continuous operation (107 injections that included 88 samples, 9 allelic ladders, and reinjections of 10 samples at ~30 min per injection)
- ~2209 sec/sample or 316 sec/genotype (~5 min/genotype)

**Mass Spectrometry Runs**

| Date | STR Loci Tested | Number of samples | Time Required for Data Collection | Average Time per Sample |
|---|---|---|---|---|
| Feb 11, 1998 | TH01 | 88 + 8 controls | 76 min for 96 samples | ~50 sec/sample |
| May 6, 1998 | TH01 | 6 samples | 2.5 min for 6 samples | ~25 sec/sample |
| October 1, 1998 | TPOX, CSF1PO, D3S1358, D7S820 | 72 + 8 controls for each locus | 60 min for 320 samples | 11.3 sec/sample |
| Jan 12, 1999 | TH01, TPOX, CSF1PO, D7S820 | 88 + 8 controls for each locus | 30 min for 384 samples | 4.7 sec/sample |
| Jan 14, 1999 | TH01, D16S539, D3S1358, Amelogenin | 88 + 8 controls for each locus | 37 min for 384 samples | 5.8 sec/sample |
| Feb 12, 1999 | D3S1358, FGA, D8S1179, DYS391 | 88 + 8 controls for each locus | 41 min for 384 samples | 6.4 sec/sample |
| March 26, 1999 | TH01, TPOX, CSF1PO, D16S539 | 88 + 8 controls for each locus | 51 min for 384 samples | 8.0 sec/sample |

Exhibit 12. **CDOJ CSF1PO results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 11,12 | 11,12 | 27,823 | 29,060 | A7 | 11,12 | 11,12 | 27,867 | 29,000 |
| B1 | 10,12 | 10,12 | 26,520 | 29,033 | B7 | 7,13 | 7,13 | 22,809 | 30,271 |
| C1 | 7,10 | 7,10 | 22,917 | 26,684 | C7 | 10,13 | 10,13 | 26,529 | 30,253 |
| D1 | 10,13 | 10,13 | 26,586 | 30,344 | D7 | 10,11 | 10,11 | 26,621 | 27,794 |
| E1 | 8,12 | 8,12 | 24,077 | 29,075 | E7 | 10,11 | 10,11 | 26,609 | 27,834 |
| F1 | 8,10 | 8,10 | 24,107 | 26,639 | F7 | 7,12 | 7,12 | 22,923 | 29,202 |
| G1 | 12,13 | 12,13 | 29,144 | 30,373 | G7 | 11,12 | 11,12 | 27,958 | 29,244 |
| H1 | 11,12 | 11,12 | 27,812 | 29,018 | H7 | 10,12 | 10,12 | 26,497 | 29,005 |
| A2 | 12,12 | 12,12 | 29,033 | | A8 | 12,12 | 12,12 | 29,129 | |
| B2 | 10,10 | 10,10 | 26,594 | | B8 | 8,11 | 8,11 | 24,117 | 27,862 |
| C2 | 10,10 | 10,10 | 26,550 | | C8 | 7,12 | 7,12 | 22,913 | 29,071 |
| D2 | 10,11 | 10,11 | 26,609 | 27,823 | D8 | 10,10 | 10,10 | 26,575 | |
| E2 | 10,12 | 10,12 | 26,620 | 29,129 | E8 | 10,12 | 10,12 | 26,552 | 29,007 |
| F2 | 10,10 | 10,10 | 26,584 | | F8 | 8,10 | 8,10 | 24,061 | 26,572 |
| G2 | 11,12 | 11,12 | 27,862 | 29,080 | G8 | 12,12 | 12,12 | 29,064 | |
| H2 | 10,10 | 10,10 | 26,605 | | H8 | 11,12 | 11,12 | 27,796 | 29,026 |
| A3 | 12,12 | 12,12 | 28,987 | | A9 | 8,12 | 8,12 | 24,085 | 29,091 |
| B3 | 10,13 | 10,13 | 26,614 | 30,380 | B9 | 11,12 | 11,12 | 27,819 | 29,022 |
| C3 | 10,10 | 10,10 | 26,618 | | C9 | 11,12 | 11,12 | 27,810 | 29,000 |
| D3 | 11,11 | 11,11 | 27,823 | | D9 | 8,13 | 8,13 | 24,093 | 30,348 |
| E3 | 11,12 | 11,12 | 27,817 | 28,998 | E9 | 11,12 | 11,12 | 27,878 | 29,098 |
| F3 | 11,12 | 11,12 | 27,871 | 29,093 | F9 | 7,12 | 7,12 | 22,836 | 29,104 |
| G3 | 10,11 | 10,11 | 26,616 | 27,812 | G9 | 9,13 | 9,13 | 25,387 | 30,389 |
| H3 | 11,12 | 11,12 | 27,849 | 29,024 | H9 | 7,11 | 7,11 | 22,866 | 27,836 |
| A4 | 8,10 | 8,10 | 24,048 | 26,499 | A10 | 9,10 | 9,10 | 25,391 | 26,607 |
| B4 | 11,13 | 11,13 | 27,941 | 30,412 | B10 | 11,12 | 11,12 | 27,844 | 29,044 |
| C4 | 11,11 | 11,11 | 27,873 | | C10 | 10,10 | 10,10 | 26,586 | |
| D4 | 10,11 | 10,11 | 26,638 | 27,794 | D10 | 8,11 | 8,11 | 24,091 | 27,838 |
| E4 | 7,10 | 7,10 | 22,835 | 26,553 | E10 | 11,12 | 11,12 | 27,843 | 29,040 |
| F4 | 10,14 | 10,14 | 26,592 | 31,565 | F10 | 10,12 | 10,12 | 26,607 | 29,111 |
| G4 | 12,14 | 12,14 | 29,113 | 31,625 | G10 | 8,11 | 8,11 | 24,029 | 27,807 |
| H4 | 9,10 | 9,10 | 25,360 | 26,582 | H10 | 8,10 | 8,10 | 24,099 | 26,609 |
| A5 | 10,10 | 10,10 | 26,590 | | A11 | 12,12 | 12,12 | 29,011 | |
| B5 | 7,11 | 7,11 | 22,818 | 27,838 | B11 | 7,12 | 7,12 | 22,836 | 29,095 |
| C5 | 10,10 | 10,10 | 26,628 | | C11 | 7,11 | 7,11 | 22,826 | 27,834 |
| D5 | 10,11 | 10,11 | 26,647 | 27,925 | D11 | 10,11 | 10,11 | 26,567 | 27,784 |
| E5 | 11,12 | 11,12 | 27,847 | 29,031 | E11 | 10,12 | 10,12 | 26,577 | 29,078 |
| F5 | 10,10 | 10,10 | 26,601 | | F11 | 10,11 | 10,11 | 26,611 | 27,825 |
| G5 | 10,12 | 10,12 | 26,603 | 29,113 | G11 | 10,10 | 10,10 | 26,605 | |
| H5 | 11,13 | 11,13 | 27,856 | 30,389 | H11 | 10,12 | 10,12 | 26,584 | 29,078 |
| A6 | 11,11 | 11,11 | 27,832 | | | | | | |
| B6 | No data | 10,12 | 26,654 | 29,180 | | | | | |
| C6 | 11,11 | 11,11 | 27,862 | | | | | | |
| D6 | 11,12 | 11,12 | 27,818 | 29,079 | | | | | |
| E6 | 11,12 | 11,12 | 27,838 | 29,049 | | | | | |
| F6 | 8,10 | 8,10 | 24,206 | 26,739 | | | | | |
| G6 | 11,12 | 11,12 | 27,843 | 29,062 | | | | | |
| H6 | 11,11 | 11,11 | 27,773 | | | | | | |

SUMMARY:

Complete agreement observed among all genotypes.

Exhibit 13. **CDOJ TPOX results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 8,10 | 8,10 | 17,914 | 20,431 | A7 | 9,9 | 5,5 | Gas-phase dimer | |
| B1 | 6,8 | 6,8 | 15,376 | 17,897 | B7 | 8,8 | 8,8 | 17,820 | |
| C1 | 9,11 | 9,11 | 19,137 | 21,663 | C7 | 10,12 | 10,12 | 20,361 | 22,830 |
| D1 | 11,12 | 11,12 | 21,562 | 22,801 | D7 | 11,12 | 6,6 | Gas-phase dimer | |
| E1 | 9,10 | 9,10 | 19,131 | 20,374 | E7 | 6,8 | 6,8 | 15,366 | 17,850 |
| F1 | 9,12 | 9,12 | 19,106 | 22,889 | F7 | 10,10 | 10,10 | 20,372 | |
| G1 | 8,8 | 8,8 | 17,838 | | G7 | 8,9 | 8,9 | 17,820 | 19,070 |
| H1 | 8,8 | 8,8 | 17,825 | | H7 | 9,9 | 9,9 | 19,098 | |
| A2 | 8,8 | 8,8 | 17,837 | | A8 | 9,10 | 5,5 | Gas-phase dimer | |
| B2 | 8,8 | 8,8 | 17,841 | | B8 | 9,9 | 5,5 | Gas-phase dimer | |
| C2 | 8,9 | 8,9 | 17,860 | 19,101 | C8 | 8,10 | 8,10 | 17,848 | 20,366 |
| D2 | 8,9 | 8,9 | 17,785 | 19,049 | D8 | 8,11 | 8,11 | 17,843 | 21,596 |
| E2 | 8,11 | 8,11 | 17,829 | 21,577 | E8 | 9,9 | 9,9 | 19,117 | |
| F2 | 8,12 | 8,12 | 17,834 | 22,816 | F8 | 9,12 | 9,12 | 19,108 | 22,793 |
| G2 | 7,10 | 7,10 | 16,525 | 20,257 | G8 | 8,8 | 8,8 | 17,905 | |
| H2 | 8,9 | 8,9 | 17,870 | 19,171 | H8 | 8,10 | 8,10 | 17,850 | 20,366 |
| A3 | 8,10 | 8,10 | 17,844 | 20,366 | A9 | 8,11 | 8,11 | 17,820 | 21,563 |
| B3 | 9,10 | 9,10 | 19,063 | 20,320 | B9 | 11,11 | 11,11 | 21,575 | |
| C3 | 9,11 | 9,11 | 19,178 | 21,676 | C9 | 8,8 | 8,8 | 17,841 | |
| D3 | 7,10 | 7,10 | 16,626 | 20,370 | D9 | 10,11 | 10,11 | 20,379 | 21,600 |
| E3 | 9,11 | 9,11 | 19,146 | 21,665 | E9 | 8,11 | 8,11 | 17,829 | 21,569 |
| F3 | 8,10 | 8,10 | 17,839 | 20,357 | F9 | 7,8 | 7,8 | 16,607 | 17,837 |
| G3 | 8,8 | 8,8 | 17,853 | | G9 | 8,10 | 8,10 | 17,904 | 20,381 |
| H3 | 6,9 | 6,9 | 15,313 | 19,070 | H9 | 8,11 | 5,5 | Gas-phase dimer | |
| A4 | 6,8 | 6,8 | 15,359 | 17,895 | A10 | 9,10 | 9,10 | 19,076 | 20,342 |
| B4 | 11,11 | 11,11 | 21,579 | | B10 | 8,11 | 8,11 | 17,827 | 21,569 |
| C4 | 8,9 | 8,9 | 17,865 | 19,126 | C10 | 8,12 | 8,12 | 17,855 | 22,850 |
| D4 | 8,9 | 8,9 | 17,839 | 19,088 | D10 | 8,9 | 8,9 | 17,846 | 19,093 |
| E4 | 6,9 | 6,9 | 15,289 | 19,070 | E10 | 9,10 | 9,10 | 19,086 | 20,346 |
| F4 | 9,11 | 5,5 | Gas-phase dimer | | F10 | 9,12 | 9,12 | 19,065 | 22,801 |
| G4 | 8,8 | 8,8 | 17,827 | | G10 | 11,12 | 11,12 | 21,554 | 22,795 |
| H4 | 8,8 | 8,8 | 17,798 | | H10 | 8,11 | 8,11 | 17,841 | 21,584 |
| A5 | 9,11 | 9,11 | 19,126 | 21,663 | A11 | 6,9 | 6,9 | 15,299 | 19,076 |
| B5 | 8,11 | 8,11 | 17,846 | 21,586 | B11 | 8,9 | 8,9 | 17,851 | 19,095 |
| C5 | 10,11 | 10,11 | 20,264 | 21,550 | C11 | 9,11 | 9,11 | 19,120 | 21,592 |
| D5 | 9,12 | 9,12 | 19,137 | 22,905 | D11 | 8,8 | 8,8 | 17,829 | |
| E5 | 9,10 | 9,10 | 19,076 | 20,337 | E11 | 11,11 | 11,11 | 21,569 | |
| F5 | 9,11 | 9,11 | 19,081 | 21,571 | F11 | 11,11 | 11,11 | 21,548 | |
| G5 | 8,8 | 8,8 | 17,831 | | G11 | 11,11 | 11,11 | 21,579 | |
| H5 | 6,11 | 5,5 | Gas-phase dimer | | H11 | 8,9 | 8,9 | 17,870 | 19,095 |
| A6 | 8,11 | 5,5 | Gas-phase dimer | | SUMMARY: | Allele | Number | Avg. mass | Std. Dev. |
| B6 | No data | 11,11 | 21,573 | | | 6 | 5 | 15,343 | 34.3 |
| C6 | 8,8 | 8,8 | 17,844 | | | 7 | 3 | 16,586 | 53.7 |
| D6 | 8,12 | 5,10 | Gas-phase dimer | | | 8 | 43 | 17,846 | 26.5 |
| E6 | 8,12 | 8,12 | 17,831 | 22,816 | | 9 | 31 | 19,102 | 33.1 |
| F6 | 8,11 | 8,11 | 17,846 | 21,600 | | 10 | 18 | 20,353 | 40.7 |
| G6 | 9,11 | 9,11 | 19,070 | 21,565 | | 11 | 24 | 21,590 | 37.9 |
| H6 | 9,10 | 9,10 | 19,151 | 20,368 | | 12 | 10 | 22,830 | 39.7 |

Exhibit 14. **CDOJ TH01 STR results compared with ABI 310 and mass spectrometry methods.** The shaded samples were run with a different primer set and have different masses.

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 7,7 | 7,7 | 18,854 | | A7 | 7,8 | 7,8 | 18,877 | 20,104 |
| B1 | 7,9.3 | 7,9.3 | 17,404 | 20,854 | B7 | 8,9.3 | 8,9.3 | 20,128 | 22,337 |
| C1 | 7,8 | 7,8 | 18,894 | 20,108 | C7 | 8,9 | 8,9 | 20,193 | 21,415 |
| D1 | 6,9 | 6,9 | 17,574 | 21,294 | D7 | 6,9.3 | 6,9.3 | 16,167 | 20,876 |
| E1 | 7,9.3 | 7,9.3 | 18,938 | 22,387 | E7 | 9.3,9.3 | 9.3,9.3 | 22,349 | |
| F1 | 9,9 | 9,9 | 21,415 | | F7 | 9,9.3 | 9,9.3 | 21,417 | 22,345 |
| G1 | 7,9 | 7,9 | 18,919 | 21,408 | G7 | 7,8 | 7,8 | 18,911 | 20,147 |
| H1 | 5,7 | 5,7 | 16,403 | 18,926 | H7 | 9,9.3 | 9,9.3 | 21,400 | 22,277 |
| A2 | 6,10 | 6,10 | 16,145 | 21,170 | A8 | 8,8 | 8,8 | 20,091 | |
| B2 | 6,6 | 6,6 | 17,662 | | B8 | 7,9.3 | 7,9.3 | 18,938 | 22,391 |
| C2 | 7,8 | 7,8 | 18,895 | 20,115 | C8 | 7,9 | 7,9 | 17,436 | 19,957 |
| D2 | 7,9 | 7,9 | 18,838 | 21,309 | D8 | 7,7 | 7,7 | 18,944 | |
| E2 | 7,9.3 | 7,9.3 | 18,904 | 22,329 | E8 | 7,7 | 7,7 | 17,376 | |
| F2 | 7,9 | 7,9 | 18,831 | 21,302 | F8 | 7,9 | 7,9 | 17,387 | 19,891 |
| G2 | 7,7 | 7,7 | 17,391 | | G8 | 9.3,9.3 | 9.3,9.3 | 22,263 | |
| H2 | 9,9.3 | 9,9.3 | 19,871 | 20,685 | H8 | 7,9.3 | 7,9.3 | 18,829 | 22,267 |
| A3 | 7,7 | 7,7 | 18,856 | | A9 | 9,9 | 9,9 | 19,907 | |
| B3 | 7,8 | 7,8 | 18,917 | 20,114 | B9 | 7,7 | 7,7 | 18,938 | |
| C3 | 7,9.3 | 7,9.3 | 17,380 | 20,828 | C9 | 9.3,9.3 | 9.3,9.3 | 22,351 | |
| D3 | 7,9 | 7,9 | 17,427 | 19,944 | D9 | 7,8 | 7,8 | 18,845 | 20,097 |
| E3 | 7,9.3 | 7,9.3 | 18,922 | 22,364 | E9 | 8,9.3 | 8,9.3 | 18,684 | 20,790 |
| F3 | 9,9.3 | 9,9.3 | 21,377 | 22,321 | F9 | 6,9.3 | 6,9.3 | 17,622 | 22,287 |
| G3 | 8,9.3 | 8,9.3 | 20,193 | 22,384 | G9 | 7,9 | 7,9 | 17,380 | 19,885 |
| H3 | 7,9.3 | 7,9.3 | 18,894 | 22,292 | H9 | 6,7 | 6,7 | 17,677 | 18,926 |
| A4 | 8,8 | 8,8 | 20,126 | | A10 | 7,8 | 7,8 | 17,389 | 18,652 |
| B4 | 6,9.3 | 6,9.3 | 17,606 | 22,294 | B10 | 9,9 | 9,9 | 21,377 | |
| C4 | 6,7 | 6,7 | 17,586 | 18,838 | C10 | 7,9 | 7,9 | 17,294 | 19,812 |
| D4 | 7,9.3 | 7,9.3 | 18,897 | 22,312 | D10 | 7,8 | 7,8 | 17,277 | 18,547 |
| E4 | 7,9 | 7,9 | 17,401 | 19,917 | E10 | 6,7 | 6,7 | 17,570 | 18,819 |
| F4 | 7,9 | 7,9 | 18,836 | 21,334 | F10 | 8,8 | 8,8 | 18,640 | |
| G4 | 8,9.3 | 8,9.3 | 20,077 | 22,258 | G10 | 6,7 | 6,7 | 17,644 | 18,906 |
| H4 | 7,9.3 | 7,9.3 | 18,899 | 22,304 | H10 | 8,9 | 8,9 | 18,643 | 19,902 |
| A5 | 7,7 | 7,7 | 17,205 | | A11 | 6,7 | 6,7 | 17,540 | 18,782 |
| B5 | 6,9 | 6,9 | 16,121 | 19,874 | B11 | 6,8 | 6,8 | 17,682 | 20,191 |
| C5 | 8,9.3 | 8,9.3 | 20,126 | 22,360 | C11 | 7,7 | 7,7 | 18,927 | |
| D5 | 6,8 | 6,8 | 17,679 | 20,184 | D11 | 7,7 | 7,7 | 17,477 | |
| E5 | 8,10 | 8,10 | 20,155 | 22,658 | E11 | 6,6 | 6,6 | 17,596 | |
| F5 | 7,9.3 | 7,9.3 | 18,870 | 22,285 | F11 | 7,8 | 7,8 | 17,400 | 18,656 |
| G5 | 6,7 | 6,7 | 17,624 | 18,906 | G11 | 8,8 | 8,8 | 18,710 | |
| H5 | 7,9 | 7,9 | 17,404 | 19,921 | H11 | 6,7 | 6,7 | 17,600 | 18,895 |
| A6 | 6,8 | 6,8 | 17,648 | 20,139 | | | | | |
| B6 | 7,7 | 7,7 | 18,910 | | | | | | |
| C6 | 8,9.3 | 8,9.3 | 18,626 | 20,823 | | | | | |
| D6 | 8,8 | 8,8 | 20,147 | | | | | | |
| E6 | 8,9.3 | 8,9.3 | 19,961 | 22,143 | | | | | |
| F6 | 8,8 | 8,8 | 20,195 | | | | | | |
| G6 | 7,9 | 7,9 | 18,858 | 21,383 | | | | | |
| H6 | 8,9 | 8,9 | 18,704 | 19,969 | | | | | |

SUMMARY:

All 88 samples were in agreement.

Exhibit 15. **CDOJ amelogenin results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | X,Y | X,Y | 25,676 | 27,566 | A7 | X,Y | X,Y | 25,774 | 27,687 |
| B1 | X,Y | X,Y | 25,730 | 27,596 | B7 | X,Y | X,Y | 25,659 | 27,555 |
| C1 | X,Y | X,Y | 25,693 | 27,667 | C7 | X,Y | X,Y | 25,797 | 27,695 |
| D1 | X,Y | X,Y | 25,686 | 27,607 | D7 | X,Y | X,Y | 25,641 | 27,533 |
| E1 | X,Y | X,Y | 25,684 | 27,765 | E7 | X,Y | X,Y | 25,674 | 27,583 |
| F1 | X,Y | X,Y | 25,693 | 27,592 | F7 | X,Y | X,Y | 25,676 | 27,585 |
| G1 | X,Y | X,Y | 25,674 | 27,574 | G7 | X,Y | X,Y | 25,668 | 27,568 |
| H1 | X,Y | X,Y | 25,653 | 27,551 | H7 | X,Y | X,Y | 25,666 | 27,564 |
| A2 | X,Y | X,Y | 25,676 | 27,574 | A8 | X,Y | X,Y | 25,659 | 27,555 |
| B2 | X,Y | X,Y | 25,674 | 27,579 | B8 | X,Y | X,Y | 25,653 | 27,544 |
| C2 | X,Y | X,Y | 25,666 | 27,559 | C8 | X,Y | X,Y | 25,668 | 27,566 |
| D2 | X,Y | X,Y | 25,768 | 27,626 | D8 | X,Y | X,Y | 25,674 | 27,566 |
| E2 | X,Y | X,Y | 25,670 | 27,568 | E8 | X,Y | X,Y | 25,843 | 27,763 |
| F2 | X,Y | X,Y | 25,657 | 27,544 | F8 | X,Y | X,Y | 25,649 | 27,581 |
| G2 | X,Y | X,Y | 25,672 | 27,646 | G8 | X,Y | X,Y | 25,661 | 27,561 |
| H2 | X,Y | X,Y | 25,641 | 27,523 | H8 | X,Y | X,Y | 25,757 | 27,585 |
| A3 | X,Y | X,Y | 25,663 | 27,559 | A9 | X,Y | X,Y | 25,641 | 27,531 |
| B3 | X,Y | X,Y | 25,770 | 27,581 | B9 | X,Y | X,Y | 25,684 | 27,579 |
| C3 | X,Y | X,Y | 25,691 | 27,635 | C9 | X,X | X,X | 25,645 | |
| D3 | X,Y | X,Y | 25,722 | 27,594 | D9 | X,Y | X,Y | 25,653 | 27,553 |
| E3 | X,Y | X,Y | 25,749 | 27,628 | E9 | X,Y | X,Y | 25,674 | 27,594 |
| F3 | X,Y | X,Y | 25,682 | 27,577 | F9 | X,Y | X,Y | 25,720 | 27,646 |
| G3 | X,Y | X,Y | 25,645 | 27,536 | G9 | X,Y | X,Y | 25,766 | 27,637 |
| H3 | X,Y | No data | | | H9 | X,Y | No data | | |
| A4 | X,Y | X,Y | 25,666 | 27,553 | A10 | X,Y | X,Y | 25,697 | 27,618 |
| B4 | X,Y | X,Y | 25,726 | 27,637 | B10 | X,Y | X,Y | 25,684 | 27,590 |
| C4 | X,Y | X,Y | 25,736 | 27,583 | C10 | X,Y | No data | | |
| D4 | X,Y | X,Y | 25,782 | 27,670 | D10 | X,Y | X,Y | 25,657 | 27,564 |
| E4 | X,Y | X,Y | 25,701 | 27,789 | E10 | X,Y | X,Y | 25,657 | 27,549 |
| F4 | X,Y | X,Y | 25,674 | 27,585 | F10 | X,Y | X,Y | 25,653 | 27,551 |
| G4 | X,Y | X,Y | 25,599 | 27,572 | G10 | X,Y | No data | | |
| H4 | X,Y | X,Y | 25,682 | 27,579 | H10 | X,Y | X,Y | 25,722 | 27,654 |
| A5 | X,Y | X,Y | 25,816 | 27,719 | A11 | X,Y | X,Y | 25,676 | 27,572 |
| B5 | X,Y | X,Y | 25,672 | 27,570 | B11 | X,Y | X,Y | 25,655 | 27,549 |
| C5 | X,Y | X,Y | 25,680 | 27,583 | C11 | X,Y | X,Y | 25,666 | 27,577 |
| D5 | X,Y | X,Y | 25,688 | 27,661 | D11 | X,Y | X,Y | 25,618 | 27,464 |
| E5 | X,Y | X,Y | 25,643 | 27,523 | E11 | X,Y | X,Y | 25,697 | 27,676 |
| F5 | X,Y | X,Y | 25,666 | 27,572 | F11 | X,Y | X,Y | 25,653 | 27,557 |
| G5 | X,Y | X,Y | 25,778 | 27,672 | G11 | X,Y | X,Y | 25,661 | 27,553 |
| H5 | X,Y | X,Y | 25,738 | 27,568 | H11 | X,Y | X,Y | 25,643 | 27,540 |
| A6 | X,Y | X,Y | 25,695 | 27,587 | | | | | |
| B6 | X,Y | X,Y | 25,649 | 27,523 | | | | | |
| C6 | X,Y | X,Y | 25,655 | 27,551 | | | | | |
| D6 | X,Y | No data | | | | | | | |
| E6 | X,Y | X,Y | 25,720 | 27,605 | | | | | |
| F6 | X,Y | X,Y | 25,674 | 27,568 | | | | | |
| G6 | X,Y | X,Y | 25,881 | 27,745 | | | | | |
| H6 | X,Y | No data | | | | | | | |

| SUMMARY: | X allele | Y allele |
|---|---|---|
| Count | 82 | 81 |
| Average mass | 25,690 Da | 27,594 Da |
| Std. dev. | 50.4 Da | 59.6 Da |

Eighty-two samples were in agreement.

"No data" resulted from mass spectrometry in six samples.

Exhibit 16. **CDOJ D3S1358 STR results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 17,17 | 17,17 | 28,182 | | A7 | 16,17 | 16,17 | 27,027 | 28,112 |
| B1 | 16,16 | 16,16 | 26,967 | | B7 | 14,19 | 14,19 | 24,305 | 30,503 |
| C1 | 15,17 | 15,17 | 25,668 | 28,139 | C7 | 14,14 | 14,14 | 24,254 | |
| D1 | 12,17 | 12,17 | 21,820 | 27,991 | D7 | 15,16 | 15,16 | 25,524 | 26,713 |
| E1 | 15,15 | 15,15 | 25,741 | | E7 | 16,16 | 16,16 | 26,822 | |
| F1 | 16,16 | 16,16 | 26,762 | | F7 | 18,18 | 18,18 | 29,491 | |
| G1 | 14,15 | 14,15 | 24,342 | 25,501 | G7 | 15,16 | 15,16 | 25,772 | 26,830 |
| H1 | 15,16 | 15,16 | 25,802 | 26,941 | H7 | 16,17 | 16,17 | 27,018 | 28,273 |
| A2 | 15,17 | 15,17 | 25,638 | 28,134 | A8 | 14,16 | 14,16 | 24,331 | 26,856 |
| B2 | 16,17 | 16,17 | 26,795 | 27,889 | B8 | 16,17 | 16,17 | 26,843 | 28,056 |
| C2 | 15,16 | 15,16 | 25,661 | 26,869 | C8 | 15,16 | 15,16 | 25,638 | 26,766 |
| D2 | 13,16 | 13,16 | 23,171 | 27,091 | D8 | 15,15 | 15,15 | 25,724 | |
| E2 | 14,17 | 14,17 | 24,352 | 28,126 | E8 | 14,15 | 14,15 | 24,343 | 25,477 |
| F2 | 15,17 | 15,17 | 25,549 | 28,091 | F8 | 15,16 | 15,16 | 25,662 | 26,703 |
| G2 | 15,17 | 15,17 | 25,641 | 28,119 | G8 | 16,16 | 16,16 | 26,907 | |
| H2 | 14,15 | 14,15 | 24,311 | 25,507 | H8 | 15,15 | 15,15 | 25,618 | |
| A3 | 17,17 | 17,17 | 28,184 | | A9 | 14,16 | 14,16 | 24,323 | 26,832 |
| B3 | 18,18 | 18,18 | 29,384 | | B9 | 15,16 | 15,16 | 25,537 | 26,739 |
| C3 | 15,15 | 15,15 | 25,645 | | C9 | 15,15 | 15,15 | 25,499 | |
| D3 | 15,15 | 15,15 | 25,645 | | D9 | 15,15 | 15,15 | 25,522 | |
| E3 | 16,17 | 16,17 | 26,967 | 27,945 | E9 | 14,15 | 14,15 | 24,440 | 25,459 |
| F3 | 15,16 | 15,16 | 25,573 | 26,790 | F9 | 16,17 | 16,17 | 26,745 | 28,021 |
| G3 | 17,18 | 17,18 | 28,025 | 29,302 | G9 | 17,18 | 17,18 | 28,372 | 29,315 |
| H3 | 14,15 | 14,15 | 24,360 | 25,530 | H9 | 15,16 | 15,16 | 25,761 | 26,777 |
| A4 | 17,17 | 17,17 | 28,001 | | A10 | 16,16 | 16,16 | 26,913 | |
| B4 | 15,16 | 15,16 | 25,518 | 26,811 | B10 | 15,18 | 15,18 | 25,539 | 29,347 |
| C4 | 15,16 | 15,16 | 25,705 | 26,722 | C10 | 15,16 | 15,16 | 25,477 | 26,741 |
| D4 | 15,17 | 15,17 | 25,589 | 28,110 | D10 | 14,15 | 14,15 | 24,214 | 25,341 |
| E4 | 16,17 | 16,17 | 27,035 | 28,023 | E10 | 14,15 | 14,15 | 24,252 | 25,507 |
| F4 | 14,16 | 14,16 | 24,374 | 26,886 | F10 | 16,17 | 16,17 | 26,871 | 28,139 |
| G4 | 14,17 | 14,17 | 24,342 | 28,115 | G10 | 16,17 | 16,17 | 27,033 | 27,934 |
| H4 | 15,18 | 15,18 | 25,655 | 29,378 | H10 | 15,16 | 15,16 | 25,716 | 26,715 |
| A5 | 15,16 | 15,16 | 25,727 | 27,110 | A11 | 15,18 | 15,18 | 25,545 | 29,293 |
| B5 | 16,17 | 16,17 | 26,988 | 28,300 | B11 | 15,15.2 | 15,15.2 | 25,734 | 26,379 |
| C5 | 15,15 | 15,15 | 25,684 | | C11 | 16,17 | 16,17 | 26,937 | 27,845 |
| D5 | 14,16 | 14,16 | 24,520 | 26,941 | D11 | 15,16 | 15,16 | 25,544 | 26,672 |
| E5 | 15,17 | 15,17 | 25,678 | 28,132 | E11 | 16,16 | 16,16 | 26,899 | |
| F5 | 16,16 | 16,16 | 26,813 | | F11 | 15,16 | 15,16 | 25,706 | 26,678 |
| G5 | 9,17 | 9,17 | 18,092 | 28,285 | G11 | 16,16 | 16,16 | 26,841 | |
| H5 | 17,17 | 17,17 | 27,893 | | H11 | 16,17 | 16,17 | 26,881 | 28,246 |
| A6 | 16,17 | 16,17 | 26,999 | 28,178 | | | | | |
| B6 | 15,17 | 15,17 | 25,645 | 28,182 | | | | | |
| C6 | 15,16 | 15,16 | 25,681 | 26,710 | | | | | |
| D6 | 15,15 | 15,15 | 25,833 | | | | | | |
| E6 | 14,16 | 14,16 | 24,301 | 26,816 | | | | | |
| F6 | 14,14 | 14,14 | 24,437 | | | | | | |
| G6 | 14,15 | 14,15 | 24,425 | 25,524 | | | | | |
| H6 | 15,15 | 15,15 | 25,523 | | | | | | |

SUMMARY:

All 88 samples were in agreement.

ABI 310 CE electropherogram of boxed data is shown in exhibit 74.

Exhibit 17. **CDOJ D16S539 STR results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 11,12 | 11,12 | 26,862 | 28,095 | A7 | 11,11 | 11,11 | 26,873 | |
| B1 | 9,14 | 9,14 | 24,421 | 30,665 | B7 | 9,10 | 9,10 | 24,338 | 25,595 |
| C1 | 9,11 | 9,11 | 24,360 | 26,888 | C7 | 11,13 | 11,13 | 26,894 | 29,471 |
| D1 | 10,11 | 10,11 | 25,622 | 26,831 | D7 | 11,11 | 11,11 | 26,879 | |
| E1 | 11,13 | 11,13 | 26,903 | 29,367 | E7 | 9,12 | 9,12 | 24,425 | 28,200 |
| F1 | 10,11 | 10,11 | 25,543 | 26,830 | F7 | 9,12 | 9,12 | 24,368 | 28,160 |
| G1 | 11,12 | 11,12 | 26,852 | 28,070 | G7 | 11,12 | 11,12 | 26,997 | 28,145 |
| H1 | 8,9 | 8,9 | 23,157 | 24,360 | H7 | 12,12 | 12,12 | 28,104 | |
| A2 | 9,11 | 9,11 | 24,342 | 26,805 | A8 | 9,13 | 9,13 | 24,360 | 29,362 |
| B2 | 12,13 | 12,13 | 28,191 | 29,323 | B8 | 9,9 | 9,9 | 24,394 | |
| C2 | 11,11 | 11,11 | 26,907 | | C8 | 9,13 | 9,13 | 24,352 | 29,369 |
| D2 | 13,13 | 13,13 | 29,445 | | D8 | 11,11 | 11,11 | 26,933 | |
| E2 | 12,12 | 12,12 | 28,128 | | E8 | 8,12 | 8,12 | 23,332 | 28,486 |
| F2 | 12,13 | 12,13 | 28,202 | 29,436 | F8 | 11,12 | 11,12 | 26,937 | 28,128 |
| G2 | 10,11 | 10,11 | 25,638 | 26,869 | G8 | 9,13 | 9,13 | 24,336 | 29,351 |
| H2 | 10,11 | 10,11 | 25,576 | 26,864 | H8 | 11,14 | 11,14 | 26,864 | 30,573 |
| A3 | 9,12 | 9,12 | 24,340 | 28,106 | A9 | 11,13 | 11,13 | 26,869 | 29,378 |
| B3 | 9,12 | 9,12 | 24,356 | 28,117 | B9 | 11,12 | 11,12 | 26,888 | 28,078 |
| C3 | 12,13 | 12,13 | 28,141 | 29,483 | C9 | 12,12 | 12,12 | 28,106 | |
| D3 | 9,9 | 9,9 | 24,417 | | D9 | 11,13 | 11,13 | 26,849 | 29,342 |
| E3 | 11,13 | 11,13 | 27,014 | 29,603 | E9 | 11,13 | 11,13 | 26,899 | 29,396 |
| F3 | 11,11 | 11,11 | 26,899 | | F9 | 12,15 | 12,15 | 28,115 | 31,845 |
| G3 | 9,11 | 9,11 | 24,376 | 26,931 | G9 | 11,13 | 11,13 | 26,886 | 29,389 |
| H3 | 8,10 | 8,10 | 23,161 | 25,649 | H9 | 11,13 | 11,13 | 26,888 | 29,387 |
| A4 | 9,11 | 9,11 | 24,427 | 26,935 | A10 | 9,11 | 9,11 | 24,423 | 26,920 |
| B4 | 9,12 | 9,12 | 24,480 | 28,248 | B10 | 9,12 | 9,12 | 24,449 | 28,126 |
| C4 | 13,13 | 13,13 | 29,318 | | C10 | 12,13 | 12,13 | 28,302 | 29,498 |
| D4 | 11,12 | No data | | | D10 | 8,9 | 8,9 | 23,072 | 24,317 |
| E4 | 13,13 | 13,13 | 29,389 | | E10 | 9,13 | 9,13 | 24,376 | 29,402 |
| F4 | 9,11 | 9,11 | 24,374 | 26,894 | F10 | 11,13 | 11,13 | 26,856 | 29,436 |
| G4 | 13,14 | No data | | | G10 | 9,9 | 9,9 | 24,358 | |
| H4 | 11,13 | 11,13 | 26,837 | 29,336 | H10 | 10,13 | 10,13 | 25,653 | 29,441 |
| A5 | 12,12 | 12,12 | 28,117 | | A11 | 9,12 | 9,12 | 24,427 | 28,128 |
| B5 | 8,9 | 8,9 | 23,181 | 24,417 | B11 | 11,12 | 11,12 | 26,862 | 28,043 |
| C5 | 10,11 | 10,11 | 25,670 | 27,007 | C11 | 11,12 | No data | | |
| D5 | 12,12 | 12,12 | 28,265 | | D11 | 11,11 | 11,11 | 26,890 | |
| E5 | 9,11 | 9,11 | 24,482 | 26,984 | E11 | 11,13 | 11,13 | 26,928 | 29,619 |
| F5 | 9,11 | 9,11 | 24,329 | 26,854 | F11 | 9,10 | No data | | |
| G5 | 10,12 | 10,12 | 25,688 | 28,222 | G11 | 10,12 | No data | | |
| H5 | 11,12 | 11,12 | 26,918 | 28,041 | H11 | 11,11 | 11,11 | 26,894 | |
| A6 | 9,11 | 9,11 | 24,372 | 26,888 | | | | | |
| B6 | No data | 9,12 | 24,405 | 28,208 | | | | | |
| C6 | 9,13 | 9,13 | 24,380 | 29,398 | | | | | |
| D6 | 9,13 | 9,13 | 24,465 | 29,416 | | | | | |
| E6 | 12,12 | 12,12 | 28,170 | | | | | | |
| F6 | 13,13 | 13,13 | 29,329 | | | | | | |
| G6 | 11,13 | 11,13 | 26,867 | 29,371 | | | | | |
| H6 | 11,11 | **9,9** | Gas-phase trimer | | | | | | |

SUMMARY:

Eighty-one samples were in agreement.

"No data" resulted from mass spectrometry in five samples.

"No data" resulted from ABI 310 in one sample.

Gas-phase trimer caused an error in one sample.

Exhibit 18. **CDOJ D7S820 STR results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 11,13 | 11,13 | 20,322 | 22,807 | A7 | 10,12 | 10,12 | 19,097 | 21,621 |
| B1 | 10,11 | 10,11 | 19,081 | 20,333 | B7 | 11,12 | 11,12 | 20,545 | 21,592 |
| C1 | 8,11 | 8,11 | 16,641 | 20,379 | C7 | 8,10 | **10,10** | 19,360 | |
| D1 | 9,10 | 9,10 | 17,857 | 19,131 | D7 | 11,12 | 11,12 | 20,383 | 21,646 |
| E1 | 8,10 | **10,10** | 19,072 | | E7 | 9,12 | 9,12 | 17,841 | 21,583 |
| F1 | 10,12 | 10,12 | 19,088 | 21,594 | F7 | 8,9 | **8,8** | 16,631 | |
| G1 | 8,10 | 8,10 | 16,643 | 19,174 | G7 | 11,12 | 11,12 | 20,374 | 21,575 |
| H1 | 8,10 | 8,10 | 16,616 | 19,088 | H7 | 11,12 | 11,12 | 20,366 | 21,581 |
| A2 | 8,11 | 8,11 | 16,646 | 20,448 | A8 | 10,12 | 10,12 | 19,189 | 21,703 |
| B2 | 10,11 | 10,11 | 19,169 | 20,381 | B8 | 10,11 | 10,11 | 19,265 | 20,400 |
| C2 | 8,8 | 8,8 | 16,534 | | C8 | 10,10 | 10,10 | 19,095 | |
| D2 | 10,10 | 10,10 | 19,189 | | D8 | 9,10 | 9,10 | 17,848 | 19,093 |
| E2 | 8,8 | 8,8 | 16,641 | | E8 | 8,10 | **10,10** | 19,169 | |
| F2 | 8,8 | 8,8 | 16,616 | | F8 | 8,9 | No data | *Double null alleles?* | |
| G2 | 8,10 | **10,10** | 19,101 | | G8 | 9,12 | 9,12 | 17,949 | 21,745 |
| H2 | 8,9 | 8,9 | 16,550 | 17,834 | H8 | 8,10 | **10,10** | 19,187 | |
| A3 | 11,12 | 11,12 | 20,372 | 21,569 | A9 | 8,11 | **11,11** | 20,571 | |
| B3 | 10,11 | 10,11 | 19,104 | 20,366 | B9 | 8,8 | 8,8 | 16,534 | |
| C3 | 8,12 | 8,12 | 16,532 | 21,548 | C9 | 10,10 | 10,10 | 19,169 | |
| D3 | 12,13 | 12,13 | 21,560 | 22,789 | D9 | 7,8 | 7,8 | 15,383 | 16,638 |
| E3 | 10,12 | 10,12 | 19,187 | 21,695 | E9 | 9,10 | 9,10 | 17,844 | 19,090 |
| F3 | 10,11 | 10,11 | 19,198 | 20,381 | F9 | 10,12 | 10,12 | 19,395 | 21,866 |
| G3 | 8,11 | **11,11** | 20,374 | | G9 | 8,10 | 8,10 | 16,651 | 19,201 |
| H3 | 8,10 | **10,10** | 19,097 | | H9 | 8,11 | 8,11 | 16,826 | 20,363 |
| A4 | 9,9 | 9,9 | 17,848 | 17,848 | A10 | 12,13 | 12,13 | 21,586 | 22,820 |
| B4 | 11,11 | 11,11 | 20,528 | | B10 | 11,12 | 11,12 | 20,387 | 21,586 |
| C4 | 8,12 | 8,12 | 16,639 | 21,667 | C10 | 8,10 | **10,10** | 19,330 | |
| D4 | 8,9 | 8,9 | 16,636 | 17,860 | D10 | 8,11 | 8,11 | 16,554 | 20,342 |
| E4 | 9,11 | **11,11** | 20,392 | | E10 | 10,11 | 10,11 | 19,086 | 20,348 |
| F4 | 10,11 | 10,11 | 19,185 | 20,396 | F10 | 9,10 | 9,10 | 17,851 | 19,097 |
| G4 | 10,10 | 10,10 | 19,101 | | G10 | 9,10 | **10,10** | 19,398 | |
| H4 | 10,12 | **12,12** | 21,579 | | H10 | 10,11 | 10,11 | 19,382 | 20,377 |
| A5 | 9,10 | 9,10 | 17,836 | 19,083 | A11 | 10,10 | 10,10 | 19,254 | |
| B5 | 11,12 | 11,12 | 20,631 | 21,678 | B11 | 10,11 | 10,11 | 19,093 | 20,353 |
| C5 | 11,12 | 11,12 | 20,363 | 21,573 | C11 | 9,10 | 9,10 | 17,886 | 19,187 |
| D5 | 10,10 | 10,10 | 19,088 | | D11 | 8,11 | 8,11 | 16,643 | 20,519 |
| E5 | 9,11 | 9,11 | 17,851 | 20,372 | E11 | 10,10 | 10,10 | 19,252 | |
| F5 | 8,11 | 8,11 | 16,634 | 20,377 | F11 | 9,12 | 9,12 | 17,831 | 21,569 |
| G5 | 11,12 | 11,12 | 20,590 | 21,787 | G11 | 10,12 | 10,12 | 19,142 | 21,653 |
| H5 | 10,10 | 10,10 | 19,322 | | H11 | 10,12 | 10,12 | 19,160 | 21,883 |
| A6 | 8,10 | 8,10 | 16,556 | 19,074 | | | | | |
| B6 | 8,9 | **8,8** | 16,643 | | | | | | |
| C6 | 8,11 | **11,11** | 20,374 | | | | | | |
| D6 | 8,10 | 8,10 | 16,614 | 19,090 | | | | | |
| E6 | 8,9 | **9,9** | 17,851 | | | | | | |
| F6 | 8,8 | 8,8 | 16,831 | | | | | | |
| G6 | 8,10 | **10,10** | 19,155 | | | | | | |
| H6 | 9,9 | 9,9 | 18,169 | | | | | | |

SUMMARY:

Seventy samples were in agreement.

"No data" resulted from mass spectrometry (null alleles?) in one sample.

Seventeen samples disagreed because of null alleles.

Exhibit 19. **CDOJ FGA STR results compared with ABI 310 and mass spectrometry methods**

| Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) | Position | ABI 310 | Mass Spec | Allele 1 (Da) | Allele 2 (Da) |
|---|---|---|---|---|---|---|---|---|---|
| A1 | 20,21 | No data | | | A7 | 23,24 | **24,24** | 40,518 | Not resolved |
| B1 | 20,23 | 20,23 | 35,987 | 39,547 | B7 | 22,25 | 22,25 | 38,290 | 41,811 |
| C1 | 23,25 | 23,25 | 39,390 | 41,917 | C7 | 24,25 | **24,24** | 41,079 | Not resolved |
| D1 | 19,23 | 19,23 | 34,858 | 39,547 | D7 | 22,22 | **21,21** | 37,930 | |
| E1 | 23,24 | **23,23** | 40,317 | Not resolved | E7 | 24,27 | 24,27 | 40,680 | 44,130 |
| F1 | 18.2,26 | 18.2,26 | 34,066 | 43,221 | F7 | 22,22 | 22,22 | 38,295 | |
| G1 | 21,21 | 21,21 | 37,011 | | G7 | 22,23 | **22,22** | 38,582 | Not resolved |
| H1 | No data | 23,23 | 39,555 | | H7 | 21,26 | 21,26 | 37,148 | 43,116 |
| A2 | 22,23 | **23,23** | 39,446 | Not resolved | A8 | OL?,24 | 16,24 | 31,415 | 40,688 |
| B2 | 21,22 | **21,21** | 37,570 | Not resolved | B8 | 22,24 | 22,24 | 38,226 | 40,874 |
| C2 | 21,25 | 21,25 | 37,146 | 41944 | C8 | 19.2,25 | 19.2,25 | 35,193 | 41,718 |
| D2 | 19,19.2 | **19,19** | 34,699 | Not resolved | D8 | 24,25 | **24,24** | 41,184 | Not resolved |
| E2 | 23,27 | **23,26** | 39,310 | 43958 | E8 | 18.2,24 | **18,23** | 33,960 | 40,247 |
| F2 | 19,25 | 19,25 | 34,667 | 41575 | F8 | 23,25 | 23,25 | 39,482 | 41,914 |
| G2 | 22,22 | 22,22 | 38,554 | | G8 | 25,25 | **24,24** | 41,668 | |
| H2 | 22,25 | 22,25 | 38,244 | 41,861 | H8 | 21,23 | 21,23 | 37,113 | 39632 |
| A3 | 22,24 | 22,24 | 38,110 | 40,604 | A9 | 19,25 | 19,25 | 34,631 | 41,827 |
| B3 | 30.2, OL? | **30,30** | 48,114 | | B9 | 20,24 | 20,24 | 35,888 | 40,688 |
| C3 | 21,24 | 21,24 | 37,158 | 40,777 | C9 | 20,24 | **19,23** | 35,667 | 40,312 |
| D3 | 23,23 | 23,23 | 39,475 | | D9 | 21,24 | 21,24 | 36,973 | 40,573 |
| E3 | 19,23 | 19,23 | 34,769 | 39,547 | E9 | 21,25 | **21,24** | 36,921 | 41,549 |
| F3 | 23,24 | **23,23** | 39,852 | Not resolved | F9 | 25,26 | **25,25** | 42,218 | Not resolved |
| G3 | 22,23 | **22,22** | 38,582 | Not resolved | G9 | 20,21 | **21,21** | 36,901 | Not resolved |
| H3 | 18.2,23 | 18.2,23 | 34,138 | 39,423 | H9 | 18.2,25 | 18.2,25 | 34,111 | 41,774 |
| A4 | 22,23 | **22,22** | 39,056 | Not resolved | A10 | 24,28 | 24,28 | 40,706 | 45,467 |
| B4 | 19,26 | 19,26 | 34,578 | 42761 | B10 | 21,24 | 21,24 | 37,018 | 40,636 |
| C4 | 24,24 | 24,24 | 40,649 | | C10 | 18,23 | 18,23 | 33,560 | 39,490 |
| D4 | 26,27 | No data | | | D10 | 22,23 | **22,22** | 38,537 | Not resolved |
| E4 | 22,22 | 22,22 | 38,442 | | E10 | 18,23 | 18,23 | 33,292 | 39,289 |
| F4 | 22,23 | **22,22** | 39,081 | Not resolved | F10 | 19,22 | 19,22 | 34,829 | 38,402 |
| G4 | 23,24 | **23,23** | 39,741 | Not resolved | G10 | 22,24 | 22,24 | 38,216 | 40,581 |
| H4 | 23,23 | 23,23 | 39,562 | | H10 | 20,26 | 20,26 | 36,127 | 43,407 |
| A5 | 20,22 | 20,22 | 35,856 | 38,364 | A11 | 24,26 | 24,26 | 40,659 | 43,043 |
| B5 | 23,23 | **22,22** | 39,017 | | B11 | 23,28 | 23,28 | 39,493 | 45,376 |
| C5 | 22,23 | **22,22** | 38,659 | Not resolved | C11 | 24,25 | **24,24** | 40,869 | Not resolved |
| D5 | 23,26 | **22,25** | 39,233 | 42,879 | D11 | 22,25 | 22,25 | 38,361 | 41,989 |
| E5 | 22,23 | **22,22** | 38,554 | Not resolved | E11 | 25,26 | **25,25** | 42,333 | Not resolved |
| F5 | 19,25 | 19,25 | 34,795 | 41,994 | F11 | 22,24 | 22,24 | 38,209 | 40,560 |
| G5 | 21,24 | 21,24 | 37,366 | 40,853 | G11 | 23,25 | 23,25 | 39,511 | 41,909 |
| H5 | 21,22 | **21,21** | 37,565 | Not resolved | H11 | 23,26 | 23,26 | 39,408 | 42,965 |
| A6 | 22,24 | 22,24 | 38,155 | 40,570 | | | | | |
| B6 | 24,27 | 24,27 | 40,612 | 44,288 | | | | | |
| C6 | 21,25 | 21,25 | 37,136 | 41,997 | | | | | |
| D6 | 20,23 | 20,23 | 35,972 | 39,477 | | | | | |
| E6 | 22,23 | **22,22** | 38,644 | Not resolved | | | | | |
| F6 | 25,28 | **25,27** | 41,737 | 45,299 | | | | | |
| G6 | 18.2,24 | 18.2,24 | 34,387 | 40,869 | | | | | |
| H6 | 21,23 | 21,23 | 37,113 | 39,534 | | | | | |

SUMMARY:

Fifty-three samples were in agreement.

"No data" resulted from mass spectrometry in two samples.

"No data" resulted from ABI 310 in one sample.

**One sample was too large for detection.**

**Nine samples resulted in wrong calls from poor calibration.**

**Twenty-two samples resulted in wrong calls from poor resolution.**