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Project Title: "High Throughput Mass Spectrometry to Exploit Genetic Differences in Same-Length STR Alleles"

Final Technical Report

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Abstract

This project focused on design, validation and utilization of a next generation STR and Y-STR typing platform based on automated electrospray ionization mass spectrometry (ESI-MS). The approach is based on using ESI-MS to "weigh" DNA forensic markers with enough accuracy to yield an unambiguous base composition (i.e. the number of A's, G's, C's and T's in an amplicon) which in turn can be used to derive an allele profile for an individual. Profiles generated are fully backwards-compatible with existing STR typing technologies.

A preliminary STR profiling assay developed during primary phase project #2006-DN-BX-K011 was refined, developmentally validated and carried into commercial manufacturing mode during the phase of this project. A 16-locus Y-STR assay was developed and brought to preliminary pilot manufacturing status. Basic developmental validation was performed and a preliminary survey of profiles from three major population groups was performed.

Because the mass spectrometer produces a measure allowing calculation of base composition rather than just length, mass spectrometric analysis shows that the standard STR markers often exhibit SNPs within the repeat region which can distinguish same length alleles. Sequence polymorphisms have been observed in 10 of 13 core CODIS STR loci, and a high percentage of polymorphic alleles have been observed in 7 of 13 core loci. An analysis of mother/father/offspring triplets demonstrated that these polymorphisms are faithfully transferred from parent to offspring and can potentially help in the resolution of certain ambiguities in relationship analysis, such as the parental source of an ambiguous parent-to-offspring germline mutation. Three of the core Y-STR loci displayed a high proportion of polymorphisms with a potentially substantial frequency variation between populations.

This project has culminated in an STR assay and associated software prepared for commercialization in the setting of a commercial organization prepared to carry the mass spectrometry-based forensics platform into mainstream application. In addition to Ibis T5000 deployments at the FBI in Quantico, VA and the University of North Texas Health Sciences Center (UNTHSC), the Ibis PLEX-ID forensics platform has recently been deployed to several locations worldwide, including AFDIL, three FBI regional state crime lab branches (New Jersey, Minnesota and Arizona), and the Australian Federal Police (AFP).

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Executive Summary

Short Tandem Repeat (STR) markers have become the human forensic "gold standard" in recent years as the combined information derived from a profile of 13 distinct alleles (CODIS 13) provide a very low probability of finding the same exact combination of alleles from two independent contributors by random chance (even two relatives, excluding identical twins). While offering extremely high differentiation, the approach is not without limitations. At low copy number it is not uncommon to observe allele "drop out" in which a heterozygous individual is typed as a homozygote because one of the alleles is not detected. Additionally, for highly degraded DNA samples, entire markers may drop out leaving only a few STRs from which to derive a DNA profile. While in some cases a partial profile can be used to include or exclude a potential suspect, there is a need within the forensics community to derive maximal information from degraded DNA samples which yield an incomplete set of STR markers.

In this project we proposed to build upon work initiated in the phase I grant #2006-DN-BX-K011, born in part out of collaborations between Ibis and both the DNA Forensics Division of the FBI (Dr. Bruce Budowle) and the Armed Forces Institute of Pathology DNA Identification Laboratory (Colonel Brion Smith, DDS, now retired) in which we have made advances developing a next-generation DNA forensics platform based on fully-automated electrospray ionization mass spectrometry (ESI-MS). The approach is based on using ESI-MS to "weigh" DNA forensic markers with enough accuracy to yield product base compositions (number of A's, G's, C's and T's). Importantly, these base composition profiles can be referenced to existing forensics databases derived from mtDNA sequence, STR, or Y-STR profiles.

We had done preliminary blinded validation studies with this approach in collaboration with both the FBI and AFIP/AFDIL to evaluate the platform for both STR and mtDNA typing. Because base compositions are used to derive specific alleles, the MS-based method picks up SNPs within STR regions that go undetected by conventional electrophoretic analyses. For example, all "allele type 11" for the D13S317 marker are not equivalent; some contain an A to T SNP which distinguish them from individuals containing the "normal" allele type 11. Individuals typed as homozygous for this allele may in fact be heterozygous, containing alleles 11 and 11 (A→T). During our phase I effort, we observed that 100% of 95 population reference samples obtained from NIST had at least one nucleotide-polymorphic allele within the core 13 CODIS STR loci.

We proposed to further develop the ESI-MS approach to STR analysis and expand the approach to the analysis of Y-chromosome STRs. We proposed to analyze sets of samples to compile nucleotide-polymorphic allele frequencies in the core CODIS STR loci and the standard forensic Y-STR loci. We also proposed to analyze samples linked by extended family relationships to verify the faithful transmission of polymorphic alleles and their utility in adding resolution to current STR typing assays. Further development and validation of this platform will yield a system that provides increased

discriminatory power while offering the cost and throughput advantages inherent to a fully automated platform.

This effort involved the development of a new technology for analysis of forensic markers and has culminated in the production of a manufactured STR kit that is currently undergoing the commercialization process, as well as production of a preliminary Y-STR research-grade kit. The specific aims, as outlined in the original application #2008-90554-CA-DN, are shown below:

Specific Aim 1 Complete the implementation of a new robust STR panel on the Ibis T5000 platform.
Specific Aim 2: Develop an ESI-MS assay for the SWGDAM-recommended Y-STR markers.

2.1 Development of a multiplex Y-STR assay
2.2 Sensitivity
2.3 Species specificity
2.4 Reproducibility and accuracy
2.5 Testing against a panel of samples / population studies

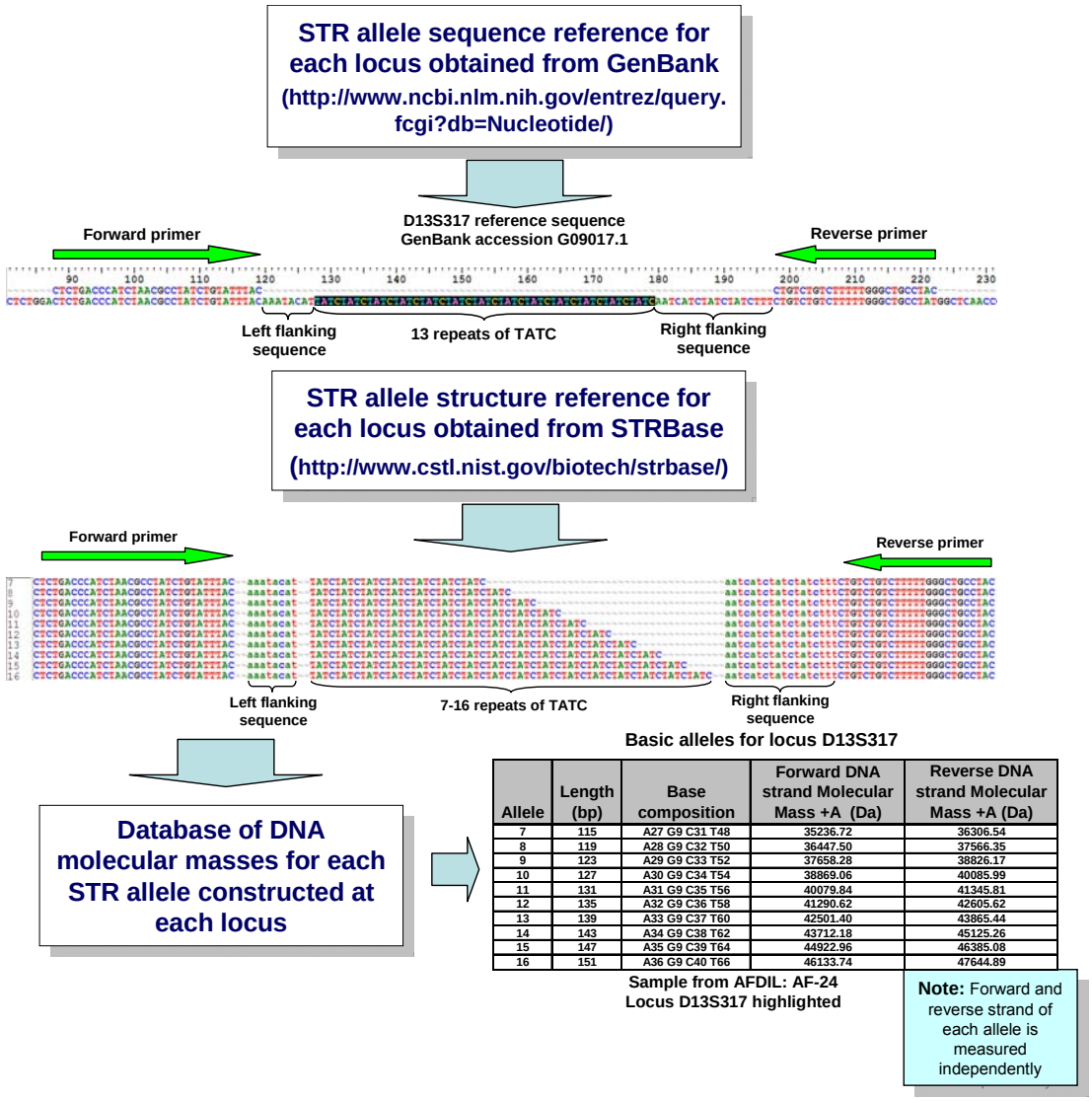
Specific Aim 3: Characterize polymorphisms in core autosomal STR and Y-STR markers
Specific Aim 4: Analysis of extended family samples.
Specific Aim 5: Continued development of transferable analysis software with an intuitive user interface

5.1 Complete the STR assay data processing automation
5.2 Refine the STR analysis interface

Specific Aim 1: Complete the implementation of a new robust STR panel on the Ibis T5000 platform.

The principle elements of our STR assay are the measurement of PCR product masses via Electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS), determination of product base compositions from their masses, and the association of the product base compositions to a database of alleles for each locus. The mass of a PCR product is an inherent property of the product that does not change according to assay conditions. Unlike measurement of product mobility in a gel, therefore, the measurement of PCR product masses does not require an allelic ladder to assign a product to the allele it represents. A database of allele base compositions serves as an electronic “ladder” that is static and precise. The basic outline of generation and use of the database in this assay is outlined in Figure 1. Accurate mass measurements reveal when an allele has a polymorphism within the amplified region relative to the reference allele because the polymorphism changes the base composition of the PCR product.

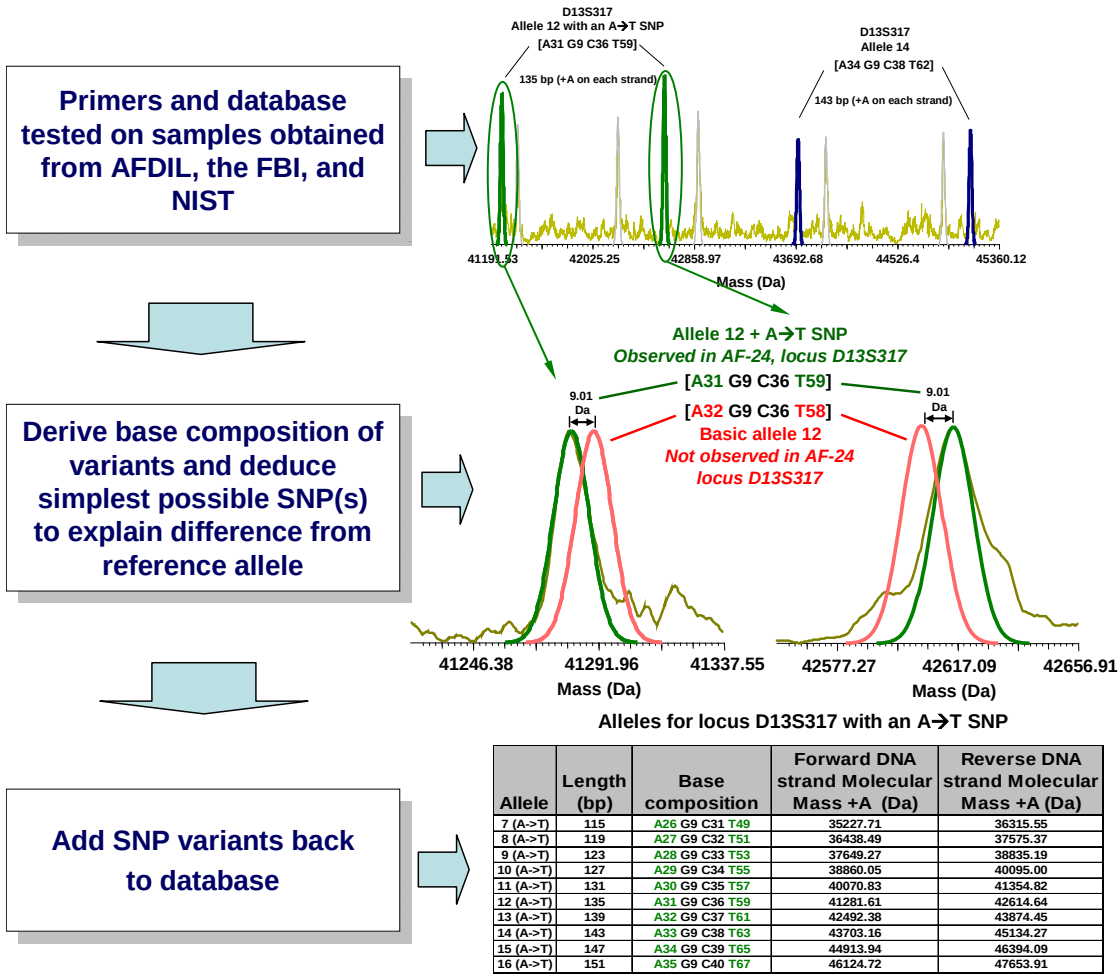
A.



Allele	Length (bp)	Base composition	Forward DNA strand Molecular Mass +A (Da)	Reverse DNA strand Molecular Mass +A (Da)
7	115	A27 G9 C31 T48	35236.72	36306.54
8	119	A28 G9 C32 T50	36447.50	37566.35
9	123	A29 G9 C33 T52	37658.28	38826.17
10	127	A30 G9 C34 T54	38869.06	40085.99
11	131	A31 G9 C35 T56	40079.84	41345.81
12	135	A32 G9 C36 T58	41290.62	42605.62
13	139	A33 G9 C37 T60	42501.40	43865.44
14	143	A34 G9 C38 T62	43712.18	45125.26
15	147	A35 G9 C39 T64	44922.96	46385.08
16	151	A36 G9 C40 T66	46133.74	47644.89

Figure 1. Panel A. The process of generating reference allele entries for an STR allele database is outlined above using D13S317 as an example.

B.



Allele	Length (bp)	Base composition	Forward DNA strand Molecular Mass +A (Da)	Reverse DNA strand Molecular Mass +A (Da)
7 (A->T)	115	A26 G9 C31 T49	35227.71	36315.55
8 (A->T)	119	A27 G9 C32 T51	36438.49	37575.37
9 (A->T)	123	A28 G9 C33 T53	37649.27	38835.19
10 (A->T)	127	A29 G9 C34 T55	38860.05	40095.00
11 (A->T)	131	A30 G9 C35 T57	40070.83	41354.82
12 (A->T)	135	A31 G9 C36 T59	41281.61	42614.64
13 (A->T)	139	A32 G9 C37 T61	42492.38	43874.45
14 (A->T)	143	A33 G9 C38 T63	43703.16	45134.27
15 (A->T)	147	A34 G9 C39 T65	44913.94	46394.09
16 (A->T)	151	A35 G9 C40 T67	46124.72	47653.91

Figure 1. Panel B. The use of an allele database in the absence of an allelic ladder. Correct allele assignments can be made by the direct measurement of product masses and the subsequent calculation of product base compositions. A polymorphism in the allele relative to the reference allele results in shifted masses of both the forward and reverse strands. Polymorphic alleles can then be added back to the database. The location of the polymorphism remains unknown unless the allele is sequenced. Also, if two cancelling polymorphisms are present (e.g. and A→G SNP and a G→A SNP within the same amplicon), the ESI-TOF-MS assay will not register a polymorphism. This is expected to be quite rare in STR alleles, however.

A mass-tagging strategy that affords great accuracy in base composition assignments has been implemented in this project (Figure 2). We have applied this strategy to our STR analyses to unambiguously assign the identity of nucleotide polymorphisms observed in STR analyses. Because an, 'A' weighs ~313.2 Da and a 'G' weighs ~329.2 Da, a base switch from an 'A' to a 'G' results in a mass shift of ~16 Da, which is very easy to measure in the mass spectrometer. However, a 'C' weighs ~289.2 Da and a 'T' weighs ~ 304.2 Da, meaning that a base switch from 'C' to 'T' results in a mass shift of ~15 Da, which is only 1 Da different than an A→G switch. Although we use base composition complementarity to assign double-stranded products, an A→G on one strand is a T→C on the complementary strand, and a C→T on one strand is a G→A on

the complementary strand, thus the complementary strands resulting from an A→G and a C→T are also 1 Da different from each other. As shown in Figure 2, the incorporation of a ^{13}C-enriched dGTP in place of normal dGTP changes the mass of one nucleotide (G) by making it ~10 Da heavier while not altering the other nucleotide masses. This mass shift results in widely-separated mass shifts for all possible combinations of base changes from any starting composition where A, G, C, T counts are each within ±10 of the starting base count. The ^{13}C-dGTP mass-tagging strategy has been fully incorporated into all STR work from PCR reaction composition to data processing and software-aided interpretation.

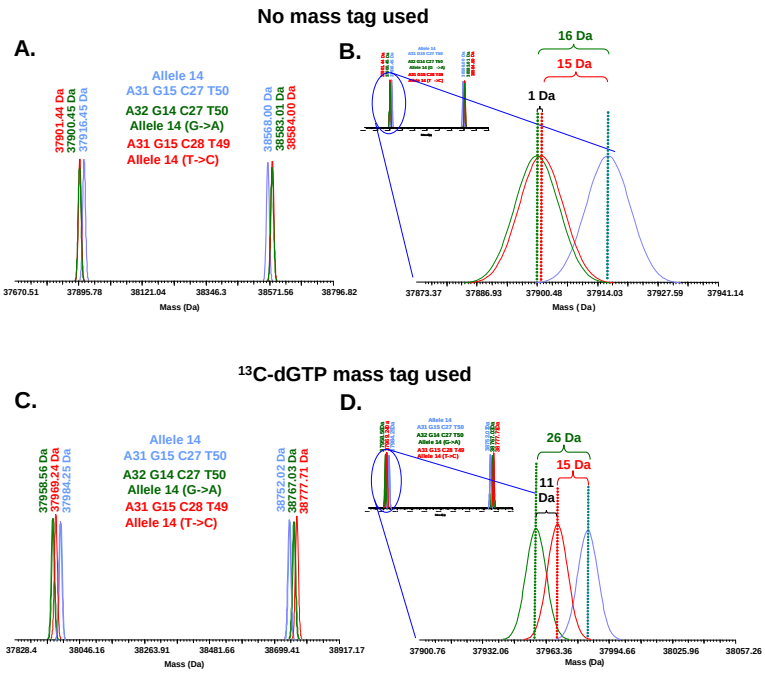


Figure 2. Use of a mass tag to make an unambiguous SNP assignment in a PCR amplicon. The example above shows locus D8S1179, allele 14, amplified with Ibis primer pair 2818. A.) Amplified with natural dNTPs, a G→A and a T→C variant produce amplicons very close in mass (about 1 Da difference). B.) Zoomed-in view of forward strand masses for allele 14 base, with a G→A, and with a T→C SNP. There is an unambiguous detection of a SNP from the base allele 14, but only a 1 Da difference between masses for G→A and T→C products, making the SNP potentially ambiguous between two possibilities. C.) When amplified with ^{13}C-enriched dGTP in place of dGTP, a G→A and a T→C variant from allele 14 produce amplicons separated by nearly 11 Da, which allows unambiguous assignment of each SNP variant. D.) Zoomed-in view of the forward strand masses for each of the three PCR products amplified with ^{13}C-enriched dGTP. There is an unambiguous detection of a SNP from the base allele 14 product, and an unambiguous assignment of the base switch involved in the SNP. The basic allele 14 product is separated from the G→A SNP by ~26 Da and from the T→C SNP by ~15 Da. The two SNP variants are separated by ~11 Da.

As outlined in the main body of the final report, as series of tests with multiple reaction conditions, primer pairs, thermocycling conditions, unrelated template DNAs, and kit stability testing has culminated in the final assay layout show in Figure 3. Thermocycling parameters have been finalized for use on the Eppendorf MasterCycler *epGradient S* and Eppendorf MasterCycler ProS thermocyclers and consist of [96°$_{10\ min}$, [96°$_{25\ sec}$, 60°$_{45\ sec}$, 72°$_{2\ min}$]$_{40\ cycles}$, 72°$_{4min}$, 96°$_{10min}$], using a 100% ramp rate for the melt-anneal transition (6°C/sec) and a 5% ramp rate for the anneal-extend transition (0.225°C/sec).

A panel of 53 unrelated DNA samples derived from human blood was prepared for developmental validation of the STR assay. Validation studies of the Ibis STR assay generally followed SWGDAM guidelines for developmental validation. Parameters listed in Table 1 were addressed as described below.

A.

Triplex 1		
PP	locus	conc
4863	CSF1PO	305
3883	D3S1358	305
3894	vWA	290

Triplex 2		
PP	locus	conc
4755	D13S317	530
1210	D16S539	220
3892	THO1	150

Triplex 3		
PP	locus	conc
3895	AMEL	200
3886	D8S1179	600
3893	TPOX	100

Triplex 4		
PP	locus	conc
3883	D3S1358	145
4866	D5S818	395
4864	D7S820	360

Triplex 5		
PP	locus	conc
1210	D16S539	140
4866	D5S818	560
3894	vWA	200

Singleplex 1		
PP	locus	conc
4451	D21S11	300

Singleplex 2		
PP	locus	conc
4976	FGA	300

Singleplex 3		
PP	locus	conc
1205	D18S51	300

B.

Primer pair number	Locus	Primer Sequences
4863	CSF1PO	TTGGCATGAAGATATTAACAGTAACTGCCTTC TCTGTGTCAGACCCTGTTCTAAGTACTTC
3883	D3S	TTGAAATCAACAGAGGCTTGCATGTAT TTGACAGAGCAAGACCCTGTCTCAT
3894	vWA	TTGGGGAGAATAATCAGTATGTGACTTGGATTG TTGGGTGATAAATACATAGGATGGATGGATAGATGG
4755	D13S317	TGGACTCTGACCCATCTAACGCCTATC TGCCATAGGCAGCCCAAAAAGACAG
1210	D16S539	TCTTCCTCTTCCCTAGATCAATACAGACAG TACCATCCATCTCTGTTTTGTCTTTCAATG
3892	THO1	TTGGAAATCAAAGGGTATCTGGGCTCTGG TTCGCTGGTCACAGGGAACACAGAC
3895	AMEL	TTGCCCTGGGCTCTGTAAAGAATAGTG TTGCATCAGAGCTTAAACTGGGAAGCTG
3886	D8S1179	TTGGGGTTTTGTATTTCATGTGTACATTCGTATC TTGGGTACCTATCCTGTAGATTATTTTCACTGTGG
3893	TPOX	TTGGCACAGAACAGGCACTTAGGGA TTGGTGTCCTTGTCAGCGTTTATTTGCC
4866	D5S818	TGGGTGATTTTCCTCTTTGGTATCCTTATGTAAT TCCAATCATAGCCACAGTTTACAACATTTG
4864	D7S820	TTGGGAACACTTGTCATAGTTTAGAACGAAC TGGCCCCTAAATGTTTACTATAGACTATTTAGTGAG
4451	D21S11	TTTTCCCAAGTGAATTGCCTTCTATC TTGAGGTAGATAGACTGGATAGATAGACGA
4976	FGA	TCCCCAGGCATATTTACAAGCTAGTTTC TGTGATTTGTCTGTAATTGCCAGCAAAAAAG
1205	D18S51	TGTGGAGATGTCTTACAATAACAGTTGCTACTA TCTGAGTGACAAATTGAGACCTTGTCTC

Figure 3. Assay layout of the finalized 14-locus Ibis STR assay. A.) Primer pair groupings and concentrations are listed. Primers were designed to minimize nontemplated adenylation and their concentrations (in triplexes) have been optimized for interlocus balance. B.) Sequences of primers in the final assay layout.

Species specificity was evaluated using a panel of nonhuman DNA: male dog and cat, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus oryzae*, ATCC and *Candida albicans*. Each non-human DNA sample was analyzed in the Ibis STR assay using 10 ng per reaction in replicates of 6. No detections were evident with exogenous templates. Mixtures of the non-human DNA with human DNA (10 ng nonhuman DNA with 1 ng human DNA) yielded full profiles for the human DNA target, with signal quality equivalent to control samples containing only human DNA.

Sensitivity was evaluated with an analysis of a dilution series of human DNA samples. A 2-fold dilution series utilizing six human DNA samples, three of which were heterozygous for all target loci in the assay, was prepared from 1 – 500 pg per reaction. All samples were analyzed in duplicate. Results are summarized in Figure 4. Increasing frequencies of missed calls became evident with ~10 genome equivalents (~60 pg) or less DNA per reaction. Full profiles were seen with 125 pg per reaction or more. An additional test was done at 50 ng per reaction to test for tolerance to high input template levels. Signal quality was equivalent to lower input levels and full profiles were obtained at 50 ng per reaction. In contrast, DNA inputs greater than approximately 1000 pg per reaction can impact the performance of ABI STR genotyping kits.

Table 1. Parameters evaluated in the developmental validation of the Ibis STR assay.

Species specificity
Sensitivity studies
Accuracy
Reproducibility
Concordance
Inheritance/population study
Positive and negative controls
Balance
Mixture studies
Assay stability

A narrower range of input DNA was analyzed to more precisely characterize the sensitivity of the assay. Dilutions of four human DNA samples were prepared at 50, 75, 100, 125, 150, and 250 or 500 pg per reaction and fifty replicates of each sample were analyzed. Results are summarized in Table 2. Frequencies of detection and calls at all DNA input levels in this range were greater than 0.97. In Table 2 the frequencies of full profile determinations are also shown. In this case the numbers of samples giving a full STR profile were determined at each input level for each DNA sample and presented as a fraction of the expected number of 50.

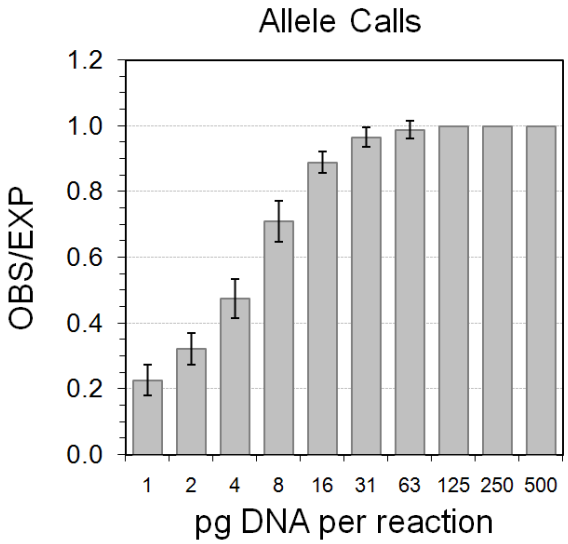


Figure 4. Broad-range sensitivity analysis of the Ibis STR assay. Eight huDNA samples were diluted as and genotyped in duplicate. The observed number of allele calls was noted for each sample and divided by the expected number of calls, then averaged across replicates and samples.

The accuracy of the assay measurements was determined by calculating the measurement error of the mass determinations made for the PCR products. The difference of the expected and observed masses of each strand detection was noted and expressed as a ratio relative to the expected mass, in units of parts per million. Data for this analysis were drawn from the sensitivity study. The average absolute measurement deviation was 11.1 ± 8.9 ppm for 39,312 independent product strand assignments. The distribution of the mass accuracy measurements for the highest input level from each of the replicates of the four human DNA samples was adequately described by the normal distribution, as has been seen with other Ibis assays.

Table 2. Sensitivity analysis of the Ibis STR assay over a narrow range of input DNA.

	Observed/Expected	
pg/well	allele calls	Samples with full profiles
250/500	0.999 ± 0.009	0.979 ± 0.017
150	0.998 ± 0.006	0.975 ± 0.030
125	0.996 ± 0.017	0.954 ± 0.031
100	0.992 ± 0.031	0.934 ± 0.025
75	0.991 ± 0.033	0.883 ± 0.029
50	0.983 ± 0.035	0.726 ± 0.073

Reproducibility was determined with data drawn from the highest DNA input levels of the sensitivity study (250 or 500 pg DNA per reaction). There were 6,242 allele detections for the four human DNA samples run in replicates of 50 at this input level. Expected allele detections were 6,248, with 6 missed detections, for detection of 99.9% of all possible alleles for this set of samples (not shown).

Table 3. Ibis STR assay genotypes for 53 blood-derived samples prepared at Ibis.

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA
55-24338	X, Y	11, 13	9, 10	10, 11	16, 17	28, 31	14 (G->A), 16 (G->A)	11, ---	8, 9	13 (A->G), 14 (A->G)	19, 20	7, 9.3	9, 11	15, 16
55-24622	X, Y	10, 12	10, 12 (A->T)	11, 12	14, 15	28, 32.2	13, 17	9 (G->T), 11	11, 12	9, 11	20, 25	7, 10	8, 11	14 (A->G + 2T->2C), 15 (G->A)
55-24336	X, Y	10, 11	11, 14	11, 12	17, ---	28, 29	15, 15 (2G->2A)	11, 12	8, 9	12, 15 (A->G)	20, 22	7, 8	8, 10	15 (G->A), 17
55-24413	X, Y	10, 12	11 (A->T), 12 (A->T)	9, 12	14, 16	29 (C->T), 31.2	14 (G->A), 16 (G->A)	11, 12	8 (C->T), 10 (T->A)	11, 13 (A->G)	19, 22	6, 9.3	8, 9	16, 18
55-24187	X, ---	10, 13	11 (A->T), ---	8, 11	14, 15	28, 31 (G->A)	14 (G->A), 18	11, 13	10, 12	10, 15 (A->G)	23, 25	9, 9.3	10, 11	15, 18
55-SMPL6	X, ---	12, 13	12 (A->T), 13	11, 12	18, 21	29, 32.2	15 (G->A), 17 (G->A)	12 (G->T), 14 (G->T)	9, 10	12, 15 (A->G)	20, 21	9.3, ---	8, ---	14 (A->G + 2T->2C), 16
55-24133	X, Y	9, 11	12 (A->T), 14	11, 12	15, 18	28, 30	14 (G->A), 16	12, 13	9, 10	14 (A->G), ---	19, 20	9.3, ---	8, ---	15 (G->A), 17 (G->A)
55-24701	X, ---	10, 12	9, 12	9, ---	14, 15	29, 32.2	16 (G->A), ---	11, 12	10, ---	15 (A->G), ---	24, 25 (C->A)	8, 9.3	8, ---	14 (A->G + 2T->2C), 16
55-24705	X, ---	11, ---	11, 12	10, 12	14, 16	27 (A->G), 31	12 (2G->2A), 17 (2G->2A)	10, 13	8, 9	13 (A->G), 14 (A->G)	22, 23	8, ---	8, 9	14 (A->G + 2T->2C), 16
55-24781	X, ---	10, 11	8, 12	11, ---	13, 16	30 (A->G), 30	15 (G->A), 17 (G->A)	12 (G->T), 12	10, ---	12, 13	22, ---	6, ---	8, 11	17, ---
55-SMPL11	X, Y	11, 12	11 (A->T), 13	11, 13	13, 19	30, 31.2	15 (G->A), ---	12, 13	11, ---	11, 14 (A->G)	21, 24	6, ---	8, ---	17, ---
SC35495	X, Y	11, 12	11 (A->T), 11	8, 9	15, 18	28, 30 (A->G)	17 (G->A), 19	11, 12	8, 9	12, 15 (A->G)	19, 23	6, 9.3	10, 11	17, 18
SC48046	X, Y	10, 11	8, 13 (A->T)	11, ---	12, 18	28, 30	15 (G->A), 17	12, 13	10, ---	13 (A->G), 15 (A->G)	23, 24	7, 9.3	8, 11	17, 19
072109B	X, Y	10, 11	12 (A->T), 12	10, 11	12, 17	31, 33.2	16 (G->A), 17 (G->A)	12 (G->T), 12	8, 10	13, 15	21, 25	6, 7	8, ---	14 (A->G + 2T->2C), 17
55-24867	X, Y	10, 12	9, 11	12, 13	16, ---	29, 32 (A->G)	15 (2G->2A), 17	12, 13 (G->C)	11, ---	13 (A->G), 16 (A->G)	24, ---	9, 9.3	11, ---	16, ---
55-24907	X, Y	10, 12	9, 12 (A->T)	11, 13	14, 17	30 (2A->2G), 32.2	15, 17 (G->A)	11, ---	9, 12	12, 14 (A->G)	21, 22	7, 9	8, 11	17, 18
55-24916	X, Y	11, ---	11 (A->T), 11	9, 12	12, 15	30 (A->G), 31.2	15 (G->A), 18 (G->A)	9 (G->T), 11	10, 12	11, 15 (A->G)	21, 24	6, 9.3	8, ---	18, 18 (G->A)
55-25006	X, Y	10, 11	8, 11 (A->T)	8, 11	13, 14	28, 31.2	15 (G->A), 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18
55-25026	X, ---	10, ---	12 (A->T), 13 (A->T)	9, 14	14, 15	29, 32.2	16, 19 (G->A)	11, 12	10, 11	14 (A->G), ---	21, 22	7, 9.3	8, 11	16, 19
55-25108	X, ---	11, 12	11 (A->T), 12	8, 11	12, 13	29, 30	16 (G->A), 16 (2G->2A)	11 (G->T), 11	9, 10 (T->A)	12, 15 (A->G)	20, 24	6, 7	8, 11	15 (G->A), 17
55-25110	X, Y	11, 13	9, 11	11, ---	13, 16	30 (A->G), 30	15 (G->A), 16	12 (G->T), 12	10, ---	12, 13	22, ---	6, 7	8, 11	18, ---
55-25113	X, Y	12, ---	11 (A->T), 12	9, 12	16, 17	29, 30 (A->G)	15 (G->A), 18	11, 12	8, 11 (T->A)	11, 12	20, 22	6, 9	8, 9	17, 19
55-25185	X, Y	10, 11	8, 12	10, 12	12, 13	29, 30 (A->G)	16, 19	11, ---	10, ---	10, 14 (A->G)	24, 25	7, ---	8, 11	18, 19
55-25188	X, Y	11, ---	8, 12 (A->T)	11, ---	14, 16	29, 30 (A->G)	14 (G->A), ---	11, 12	10 (T->A), 12	10, 14 (A->G)	20, 22	6, 7	8, ---	17, ---
55-25192	X, Y	10, 11	11, 12 (A->T)	11, 13	13, 19	31.2, 32.2	15 (G->A), 18	13 (G->T), 13	8, 9	12, 13 (A->G)	23, 25	6, 7	8, ---	16 (G->A), 17
55-25193	X, Y	10, 11	8, 12 (A->T)	9, 11	14, 16	30 (A->G), 30.2 (G->A)	15 (G->A), 17	12 (G->T), 12	12 (T->A), 13	12, 14 (A->G)	20, 22	6, 7	8, ---	18, 19
55-25236	X, Y	10, 12	11, ---	12, ---	13, 15	29, 30	17 (G->A), 18	11, ---	11, 13	13 (A->G), 14	21, 22	9.3, ---	8, ---	14 (A->G + 2T->2C), 16
55-25238	X, Y	10, 11	11, 12 (A->T)	12, 14	12, 22	30 (A->G), 30	16 (G->A), ---	11, 12	9, 10	13 (A->G), 15 (A->G)	19, 23	6, 8	8, ---	16, 18
55-25290	X, Y	11, ---	11, 12	9, 13	13, ---	30, 32.2	18, ---	11, 12 (G->T)	8, 11 (T->A)	14 (A->G), ---	21, 25	8, 9.3	8, ---	17, 20
55-25295	X, ---	11, ---	11, 12	10, 12	14, 16	27 (A->G), 31	12 (2G->2A), 17 (2G->2A)	10, 13	8, 9	13 (A->G), 14 (A->G)	22, 23	8, ---	8, 9	14 (A->G + 2T->2C), 16
55-25307	X, Y	10, 12	11, 11 (A->T)	10, 11	13, 18	33.2, ---	14 (G->A), 16	11 (G->T), 12	7, 8	13, 14 (A->G)	21, 24	6, ---	8, 11	16, 17
55-25356	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
55-25364	X, Y	10, 12	8, 11 (A->T)	12, ---	13, 16	29, 30	16 (2G->2A), 16 (G->A)	12 (G->T), 13	11, ---	10, ---	24, 28.1	6, 8	8, 11	17, 19
55-25367	X, Y	11, 12	12, 12 (A->T)	12, ---	17, ---	28, 31	15 (G->A), 17 (G->A)	9 (G->T), 10 (G->T)	10, 11	15 (A->G), ---	22, 25	6, 9.3	8, 12	18, 20
55-25378	X, ---	11, 12	10, 11	12, 13	13, 14	28, 30	14 (G->A), 17 (G->A)	10 (G->T), 13	9, 10	9, 13 (A->G)	23, ---	6, ---	8, 10	16, 17
55-25380	X, Y	10, 11	12, 13 (A->T)	9, 12	12, 17	29, ---	15 (G->A), 16 (2G->2A)	12 (G->T), 13	9, 10	14 (A->G), 16 (2A->2G)	22, 25	6, 8	11, ---	16, 18
55-25381	X, Y	11, 12	8, 11 (A->T)	9, 13	14, 17	27 (A->G), 30.2	16, 17	11, 12 (G->T)	10, ---	12, 13	19, 23	8, 9	8, 11	14 (T->C), 18
55-25445	X, Y	11, 14	8, 12	9, 11	12, 15	28, 31.2	15 (G->A), ---	11, 12	7, 9	11, 15 (A->G)	20, 22	9, 9.3	8, ---	17, ---
55-25446	X, ---	10, 12	12 (A->T), 13	11, 12	13, 14	28, 29	17 (G->A), 18	12, 13	11, ---	12, 15 (A->G)	22.2, 23	6, 7	10, 11	15, 16
55-25456	X, Y	8, 9	12, ---	10, 13	17, 18	28, 29	15 (2G->2A), 17 (2G->2A)	12, 13	8, ---	13 (A->G), 16 (A->G)	23, 24	6, 8	9, 11	15 (G->A), 16
55-25460	X, Y	11, 13	8, 12 (A->T)	9, 13	14, ---	28, ---	16 (G->A), 17 (G->A)	10, 13	10, 11	11, 13 (A->G)	19, 20	8, 9.3	9, 11	17, 18
55-25461	X, ---	11, 12	12 (A->T), ---	9, 11	12, 14	28, 29	15 (G->A), ---	11, 12 (G->T)	10, ---	14 (A->G), ---	21, 24	6, 7	10, 12	14 (T->C), 15 (G->A)
55-25462	X, Y	11, 13	11, 12 (A->T)	11, ---	12, 13	27 (A->G), 28	15 (G->A), 16 (G->A)	12, 13 (G->T)	9, 13	14 (A->G), 15 (A->G)	21, 24	6, 7	8, 11	15 (G->A), 16
55-25502	X, Y	10, 12	9, 12 (A->T)	11, 13	14, 17	30 (2A->2G), 32.2	15, 17 (G->A)	11, ---	9, 12	12, 14 (A->G)	21, 22	7, 9	8, 11	17, 18
55-25577	X, Y	10, 11	8, 11 (A->T)	8, 11	13, 14	28, 31.2	15 (G->A), 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18
55-25578	X, Y	10, 13	8, 12 (A->T)	11, 13	11, 16	27, 28	14 (G->A), 15 (G->A)	11, 13	8, 10	12 (A->G), 15 (A->G)	19, 21	6, 9	8, 11	18, 19
55-25597	X, Y	10, ---	11, 11 (A->T)	11, 13	12, 13	30 (A->G), 32.2	16 (G->A), 18	11, 13	9, 10	13 (A->G), 14	20, 25	9.3, 10	11, 12	17, 18
55-25600	X, Y	11, ---	11 (A->T), 12 (A->T)	9, 12	14, 15	30, 30 (A->G)	16, 16 (G->A)	8, 13 (G->T)	8, 10 (T->A)	13 (A->G), 16 (A->G)	20, 24	6, ---	8, 11	18, ---
55-25602	X, Y	11, 12	12, 14	12, 13	12, 16	30, 32.2	15 (G->A), 16 (G->A)	10, 11	12, 13 (T->A)	11, 13 (A->G)	22, 24	6, 9.3	8, 11	14 (A->G + 2T->2C), 16
55-25603	X, Y	10, 12	11, 12 (A->T)	9, 11	13, 15	29, 30	15 (G->A), 16 (G->A)	11, 12	10, 11	12, 16 (A->G)	21, ---	9, 9.3	8, 11	16, ---
55-25704	X, ---	11, 12	10, 12 (A->T)	11, 12	10, 16	25 (3A->3G), 29	17, ---	11 (G->T), 12	8, 10 (T->A)	12, 14 (A->G)	20, 21	6, 9	10, ---	16, 17
55-25705	X, Y	10, 11	12, 13	10, 11	16, 19	29, 30.2	16, 17 (G->A)	13, 13 (G->C)	8, 11	13 (A->G), 15 (A->G)	19, 24	7, ---	11, ---	17, 20 (A->G + A->G)
55-25711	X, ---	10, 12	9, 13	12, ---	12, 14	28, 30	15 (2G->2A), 16 (G->A)	11, 12	8, 11	10, 13 (A->G)	21, 25	6, ---	8, 11	16 (G->A), 18

Table 4. Identifiler assay genotypes for 53 blood-derived samples prepared at Ibis.

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA	D19S433	D2S1338
55-24338	X, Y	11, 13	9, 10	10, 11	16, 17	28, 31	14, 16	11, ---	8, 9	13, 14	19, 20	7, 9.3	9, 11	15, 16	13, 14	17, 19
55-24622	X, Y	10, 12	10, 12	11, 12	14, 15	28, 32.2	13, 17	9, 11	11, 12	9, 11	20, 25	7, 10	8, 11	14, 15	12, 13	17, 18
55-24336	X, Y	10, 11	11, 14	11, 12	17, ---	28, 29	15, ---	11, 12	8, 9	12, 15	20, 22	7, 8	8, 10	15, 17	14.2, ---	17, 19
55-24413	X, Y	10, 12	11, 12	9, 12	14, 16	29, 31.2	14, 16	11, 12	8, 10	11, 13	19, 22	6, 9.3	8, 9	16, 18	14, 15	17, 19
55-24187	X, ---	10, 13	11, ---	8, 11	14, 15	28, 31	14, 18	11, 13	10, 12	10, 15	23, 25	9, 9.3	10, 11	15, 18	14, ---	23, 24
55-SMPL6	X, ---	12, 13	12, 13	11, 12	18, 21	29, 32.2	15, 17	12, 14	9, 10	12, 15	20, 21	9.3, ---	8, ---	14, 16	15, 16.2	19, 24
55-24133	X, Y	9, 11	12, 14	11, 12	15, 18	28, 30	14, 16	12, 13	9, 10	14, ---	19, 20	9.3, ---	8, ---	15, 17	14, 15.2	17, 19
55-24701	X, ---	10, 12	9, 12	9, ---	14, 15	29, 32.2	16, ---	11, 12	10, ---	15, ---	24, 25	8, 9.3	8, ---	14, 16	12, 14	16, 23
55-24705	X, ---	11, ---	11, 12	10, 12	14, 16	27, 31	12, 17	10, 13	8, 9	13, 14	22, 23	8, ---	8, 9	14, 16	15, ---	18, 19
55-24781	X, ---	10, 11	8, 12	11, 12	12, 13	29, 30	17, 18	10, 13	11, ---	14, ---	23, ---	7, 9.3	8, ---	16, 19	13, 15	17, 22
55-SMPL11	X, Y	11, 12	11, 13	11, 13	13, 19	30, 31.2	15, ---	12, 13	11, ---	11, 14	21, 24	6, ---	8, ---	17, ---	14, ---	17, 25
SC35495	X, Y	11, 12	11, ---	8, 9	15, 18	28, 30	17, 19	11, 12	8, 9	12, 15	19, 23	6, 9.3	10, 11	17, 18	12, 15	25, 26
SC48046	X, Y	10, 11	8, 13	11, ---	12, 18	28, 30	15, 17	12, 13	10, ---	13, 15	23, 24	7, 9.3	8, 11	17, 19	13, 14	22, 25
072109B	X, Y	10, 11	12, ---	10, 11	12, 17	31, 33.2	16, 17	12, ---	8, 10	13, 15	21, 25	6, 7	8, ---	14, 17	12, 15	25, ---
55-24867	X, Y	10, 12	9, 11	12, 13	16, ---	29, 32	15, 17	12, 13	11, ---	13, 16	24, ---	9, 9.3	11, ---	16, ---	12.2, 14	23, 25
55-24907	X, Y	10, 12	9, 12	11, 13	14, 17	30, 32.2	15, 17	11, ---	9, 12	12, 14	21, 22	7, 9	8, 11	17, 18	14, ---	17, 19
55-24916	X, Y	11, ---	11, ---	9, 12	12, 15	30, 31.2	15, 18	9, 11	10, 12	11, 15	21, 24	6, 9.3	8, ---	18, ---	12, 13	19, ---
55-25006	X, Y	10, 11	8, 11	8, 11	13, 14	28, 31.2	15, 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18	14, 15.2	19, 25
55-25026	X, ---	10, ---	12, 13	9, 14	14, 15	29, 32.2	16, 19	11, 12	10, 11	14, ---	21, 22	7, 9.3	8, 11	16, 19	14, 15	20, 23
55-25108	X, ---	11, 12	11, 12	8, 11	12, 13	29, 30	16, ---	11, ---	9, 10	12, 15	20, 24	6, 7	8, 11	15, 17	13, 14	23, 25
55-25110	X, Y	11, 13	9, 11	11, ---	13, 16	30, ---	15, 16	12, ---	10, ---	12, 13	22, ---	6, 7	8, 11	18, ---	14, ---	17, 25
55-25113	X, Y	12, ---	11, 12	9, 12	16, 17	29, 30	15, 18	11, 12	8, 11	11, 12	20, 22	6, 9	8, 9	17, 19	12, 16	22, 23
55-25185	X, Y	10, 11	8, 12	10, 12	12, 13	29, 30	16, 19	11, ---	10, ---	10, 14	24, 25	7, ---	8, 11	18, 19	14, ---	17, ---
55-25188	X, Y	11, ---	8, 12	11, ---	14, 16	29, 30	14, ---	11, 12	10, 12	10, 14	20, 22	6, 7	8, ---	17, ---	14, ---	17, 24
55-25192	X, Y	10, 11	11, 12	11, 13	13, 19	31.2, 32.2	15, 18	13, ---	8, 9	12, 13	23, 25	6, 7	8, ---	16, 17	12, 15.2	20, 25
55-25193	X, Y	10, 11	8, 12	9, 11	14, 16	30, 30.2	15, 17	12, ---	12, 13	12, 14	20, 22	6, 7	8, ---	18, 19	13, 14	18, 23
55-25236	X, Y	10, 12	11, ---	12, ---	13, 15	29, 30	17, 18	11, ---	11, 13	13, 14	21, 22	9.3, ---	8, ---	14, 16	14, ---	20, 25
55-25238	X, Y	10, 11	11, 12	12, 14	12, 22	30, ---	16, ---	11, 12	9, 10	13, 15	19, 23	6, 8	8, ---	16, 18	12, 14	17, 21
55-25290	X, Y	11, ---	11, 12	9, 13	13, ---	30, 32.2	18, ---	11, 12	8, 11	14, ---	21, 25	8, 9.3	8, ---	17, 20	15.2, 16	19, 23
55-25295	X, ---	11, ---	11, 12	10, 12	14, 16	27, 31	12, 17	10, 13	8, 9	13, 14	22, 23	8, ---	8, 9	14, 16	15, ---	18, 19
55-25307	X, Y	10, 12	11, ---	10, 11	13, 18	33.2, ---	14, 16	11, 12	7, 8	13, 14	21, 24	6, ---	8, 11	16, 17	12, 15.2	23, 26
55-25356	X, Y	10, 12	8, 10	11, 12	15, 17	27, 29	17, 18	10, 13	8, 11	13, ---	22, 25	7, 9	8, 11	15, 18	14, 15	17, 24
55-25364	X, Y	10, 12	8, 11	12, ---	13, 16	29, 30	16, ---	12, 13	11, ---	10, ---	24, OL*	6, 8	8, 11	17, 19	15.2, ---	16, 18
55-25367	X, Y	11, 12	12, ---	12, ---	17, ---	28, 31	15, 17	9, 10	10, 11	15, ---	22, 25	6, 9.3	8, 12	18, 20	14, 16	20, 23
55-25378	X, ---	11, 12	10, 11	12, 13	13, 14	28, 30	14, 17	10, 13	9, 10	9, 13	23, ---	6, ---	8, 10	16, 17	12, 14	17, 20
55-25380	X, Y	10, 11	12, 13	9, 12	12, 17	29, ---	15, 16	12, 13	9, 10	14, 16	22, 25	6, 8	11, ---	16, 18	13, 13.2	16, 19
55-25381	X, Y	11, 12	8, 11	9, 13	14, 17	27, 30.2	16, 17	11, 12	10, ---	12, 13	19, 23	8, 9	8, 11	14, 18	12, 14	19, ---
55-25445	X, Y	11, 14	8, 12	9, 11	12, 15	28, 31.2	15, ---	11, 12	7, 9	11, 15	20, 22	9, 9.3	8, ---	17, ---	13, 15	24, 25
55-25446	X, ---	10, 12	12, 13	11, 12	13, 14	28, 29	17, 18	12, 13	11, ---	12, 15	22.2, 23	6, 7	10, 11	15, 16	15, 15.2	17, 25
55-25456	X, Y	8, 9	12, ---	10, 13	17, 18	28, 29	15, 17	12, 13	8, ---	13, 16	23, 24	6, 8	9, 11	15, 16	14, 16.2	22, 23
55-25460	X, Y	11, 13	8, 12	9, 13	14, ---	28, ---	16, 17	10, 13	10, 11	11, 13	19, 20	8, 9.3	9, 11	17, 18	14, 16	17, ---
55-25461	X, ---	11, 12	12, ---	9, 11	12, 14	28, 29	15, 17	11, 12	10, ---	10, 14	21, 24	6, 7	10, 12	14, 15	13, 16	20, 26
55-25462	X, ---	11, 13	11, 12	11, ---	12, 13	27, 28	15, 16	12, 13	9, 13	14, 15	21, 24	6, 7	8, 11	15, 16	14, 16	21, 23
55-25502	X, Y	10, 12	9, 12	11, 13	14, 17	30, 32.2	15, 17	11, ---	9, 12	12, 14	21, 22	7, 9	8, 11	17, 18	14, ---	17, 19
55-25577	X, Y	10, 11	8, 11	8, 11	13, 14	28, 31.2	15, 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18	14, 15.2	19, 25
55-25578	X, Y	10, 13	8, 12	11, 13	11, 16	27, 28	14, 15	11, 13	8, 10	12, 15	19, 21	6, 9	8, 11	18, 19	13, 14	20, 24
55-25597	X, Y	10, ---	11	11, 13	12, 13	30, 32.2	16, 18	11, 13	9, 10	13, 14	20, 25	9.3, 10	11, 12	17, 18	13, 15	17, 23
55-25600	X, Y	11, ---	11, 12	9, 12	14, 15	30, ---	16, ---	8, 13	8, 10	13, 16	20, 24	6, ---	8, 11	18, ---	12, 14	17, 23
55-25602	X, Y	11, 12	12, 14	12, 13	12, 16	30, 32.2	15, 16	10, 11	12, 13	11, 13	22, 24	6, 9.3	8, 11	14, 16	14, 16	23, 25
55-25603	X, Y	10, 12	11, 12	9, 11	13, 15	29, 30	15, 16	11, 12	10, 11	12, 16	21, ---	9, 9.3	8, 11	16, ---	12, 13	19, 21
55-25704	X, ---	11, 12	10, 12	11, 12	10, 16	25, 29	17, ---	11, 12	8, 10	12, 14	20, 21	6, 9	10, ---	16, 17	12, 13	19, 20
55-25705	X, Y	10, 11	12, 13	10, 11	16, 19	29, 30.2	16, 17	13, ---	8, 11	13, 15	19, 24	7, ---	11, ---	17, 20	13.2, 14	17, 24
55-25711	X, ---	10, 12	9, 13	12, ---	12, 14	28, 30	15, 16	11, 12	8, 11	10, 13	21, 25	6, ---	8, 11	16, 18	12, 14	24, 25

Concordance was evaluated by analyzing a panel of 53 human DNA samples in parallel with the Ibis STR assay and with the ABI Identifiler assay. Results generated with the Ibis and AB Identifiler™ assays are shown in Tables 3 and 4, respectively. All

samples were analyzed using 1 ng DNA per well. Profiles determined with the Ibis STR assay were made backwards-compatible with Identifiler profiles by using base allele calls, ignoring the SNP-based polymorphisms detected with the Ibis assay. There was 100% concordance of the Ibis STR profiles with the Identifiler assay.

Accurate mass determinations made with the Ibis STR assay enable the routine identification of SNPs in the target loci. In the course of the development of the Ibis STR assay the occurrence of STR SNP variants and their inheritance in family sample sets have been examined over multiple sample sets. These studies were done in collaboration with John Planz and Art Eisenberg of the University of North Texas Health Sciences Center (UNTHSC), Fort Worth, TX, John Butler at NIST, and Cecelia Crouse at the Palm Beach County Sherriff's Office (Palm Beach, FL). The data are referenced here in support of the developmental validation of the Ibis STR assay, specifically with regard to SNP detections.

A preliminary determination of the frequency of SNP variants in the CODIS loci made with a panel of DNA samples derived from 297 Caucasian, 332 African American, and 313 Hispanic individuals is presented in the main report body. SNP polymorphisms were observed in all assay loci except AMEL, THO1, and TPOX, with a high frequency of SNPs being observed in seven of 13 autosomal loci. Results appear in Table 5.

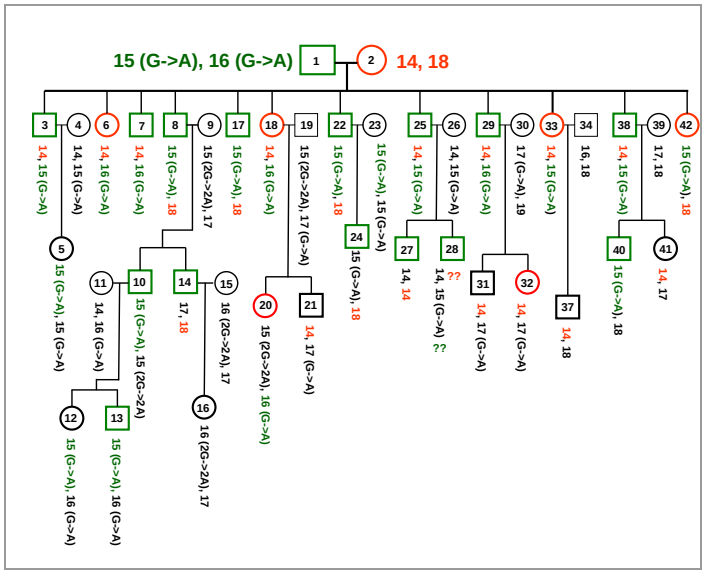


Figure 5. Inheritance of D3S1358 alleles within a 42-sample pedigree set. Genotypes were obtained for each of 42 samples with the Ibis STR assay. Passage of the D3S1358 alleles is illustrated above, with paternal alleles (sample 1) colored green, maternal alleles (sample 2) colored red, and alleles originating from outside of the primary pedigree colored black.

SNP assignments could be informative in situations benefitting from additional discriminatory power, such as where partial profiles are obtained, or with analysis of inheritance. Figure 5 shows an example of the passage of D3S1358 alleles through a family of 42 individuals. SNPs were evident in both D3S1358 alleles of the grandfather (sample 1), and consequently all of his children acquired one of these two SNP variants. Notably, a grandchild (sample 10) acquired allele 15 (G→A) from his grandfather, together with allele 15 (2G→2A) from outside of the primary pedigree. This individual would be typed as homozygous with conventional STR typing methods, but with the Ibis STR assay he was identified as heterozygous at this locus, with allele 15 (G→A) derived from the primary pedigree.

Trio samples potentially having germline mutations transmitted from parent to offspring were identified with conventional STR typing methods by our UNTHSC collaborators. A panel of these samples was with the Ibis STR assay using a subset of

loci showing the highest frequencies of SNPs: D13S317, D21S11, D3S1358, D5S818, D7S820, D8S1179, and vWA and results are presented in the main report body.

Ibis assays are configured in a 96-well plate format and are fabricated in a highly automated process. During the term of this NIJ contract the production of the STR assay was scaled up from benchtop production of 10-20 plates to fabrication of 200 plates in the Ibis Pilot Manufacturing Suite. Production of the STR kit had been transferred to the Ibis Manufacturing group, and three kit production runs have been completed with 200-500 plates produced per run. As of September 2010 the Ibis Manufacturing group projected production of 2,920 plates to meet existing commitments for the year 2011.

Implicit in the transfer of the assay to Manufacturing has been the development of quality control metrics and release specifications for the production and lot testing of kitted product. With commercialization the assay falls under the QA/QC policies and procedures in place at Ibis Biosciences and Abbott Molecular, and documentation of the production and further development of the assay from a QA/QC standpoint also is consistent with the needs of the forensic community. A panel of human DNA samples is used to track assay performance during the kitting process, and the release of a manufactured lot is dependent on analysis specifications of the panel as well.

Specific Aim 2: Develop an ESI-MS assay for the SWGDAM-recommended Y-STR markers.

Following the approach for developing an automated assay for autosomal STR markers, an assay has been developed that targets the 16 Y-STR loci and has the same general layout as the Ibis STR assay (each sample occupies one column of a 96-well plate, Figure 6). Targeted loci are DYS393, DYS19, DYS391, DYS389I/II, DYS390, DYS385a/b, DYS392, DYS437, DYS438, DYS439, DYS456, DYS458, DYS635, Y-GAT-H4 and DYS448. Information required to perform Y-STR analyses fits directly into our current allele-based genotyping system.

A.

Primer Pair Number	Locus	Primer sequences
4579	DYS19	TGCCTACTGAGTTTCTGTTATAGTGTTTTTT TCATCTGGGTTAAGGAGAGTGTCAC
4692	DYS385a/b	TTTAAAGAGAAAGAGGAAAGAGAAAGAAAGG TGTGGGATAATCTATCTATTCCAATTACATAGTC
4586	DYS389I	TCCAACTCTCATCTGTATTATCTATGTATCTG TAGATAGATTGATAGAGGGAGGGATAGATAG
4587	DYS389II-1	TCCAACTCTCATCTGTATTATCTATGTGTG TGATGAGAGTTGGATACAGAAGTAGGTATAATG
4591	DYS390	TCTGCATTTTGGTACCCCATAATATATTC TGTGTATACTCAGAAACAAGGAAAGATAGATA
4594	DYS391	TCATTCAATCATACACCCATATCTGTCTG TGCAAGCAATTGCCATAGAGG
4597	DYS392	TGGAAAACAAATTTTTTCCTTGTATCACCA TCCATTAAACCTACCAATCCCATTCC
4601	DYS393	TGGTGGTCTTCTACTTGTGTCAATAC TGGAACTCAAGTCCAAAAATGAGG
4608	DYS437	TCGTGAGTGCATGCCCATC TCGTTCATAGATAAGTAGATAGACATCATTCAC
4611	DYS438	TCCAAAATTAGTGGGGAATAGTTGAACG TAGCCTGGGCAACAAGAGTG
4615	DYS439	TAGATAGATACATAGGTGGAGACAGATAGATG TGCTTGGAATTCTTTTACCCATCATCTC
4902	DYS448	TGAGACAGAAAGGGAGATAGAGACATGG TATTTCTGGCCGGTCTGGAAATTTATCTC
5157	DYS456	TGTGGGACCTTGTGATAATGTAAGATAGATAG TTCTCTAGAGGGACAGAACTAATGGAATATC
4924	DYS458	TCTGAGCAACAGGAATGAAACTC TGAGCCACCACGCCCAC
5139	DYS635	TCCCCGGATAAAGAAAATGTGATAGATAGATAG TCTTGGCTTCTCACTTTGCATAGAATC
4912	Y-GATA-H4	TCCGCTGAGGAGAATTTCCAAATTTAAG TCCCCTATCTATCTATTCATCCATCTAATCTATCC

B.

Reaction 1		
pp	locus	conc
4692	DYS385a/b	500

Reaction 2		
pp	locus	conc
4902	DYS448	500

Reaction 3		
pp	locus	conc
4591	DYS390	250

Reaction 4		
pp	locus	conc
4587	DYS389II-1	315
4601	DYS393	200

Reaction 5		
pp	locus	conc
4611	DYS438	500
5139	DYS635	300

Reaction 6		
pp	locus	conc
4586	DYS389I	150
4594	DYS391	225
4924	DYS458	400

Reaction 7		
pp	locus	conc
4579	DYS19	400
4608	DYS437	100
4912	Y-GATA-H4	200

Reaction 8		
pp	locus	conc
4597	DYS392	150
4615	DYS439	300
5157	DYS456	330

C.

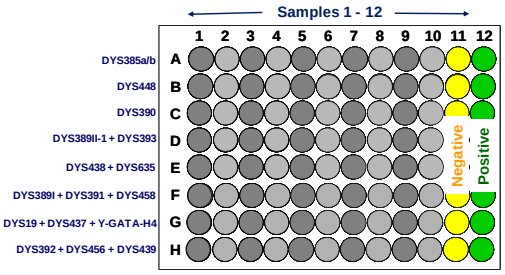


Figure 6. Primer pairs, concentrations and assay layout for 8-well Y-STR assay covering 16 loci. A.) The primer pairs for each of the 16 loci covered in the current assay. B.) Primer pair reaction combinations and concentrations used in final reactions (in nM). C.) Intended assay layout on a 96-well plate.

Pre-fabricated PCR plates are heat-sealed and frozen at -20 °C prior to use in thermocycling. Plate setup consists of pipetting 5 μL of purified DNA template into the 8 wells of one column on the assay plate for each sample, resealing the plate, and thermocycling. After thermocycling, the plate is set directly on the T5000 or PLEX-ID instrument and all downstream steps up to final analysis and data QC are automated. Presently, Manual pilot kit runs have been produced in batches of 50 plates. This assay has not currently been fully transferred to the Ibis manufacturing facility. Preliminary

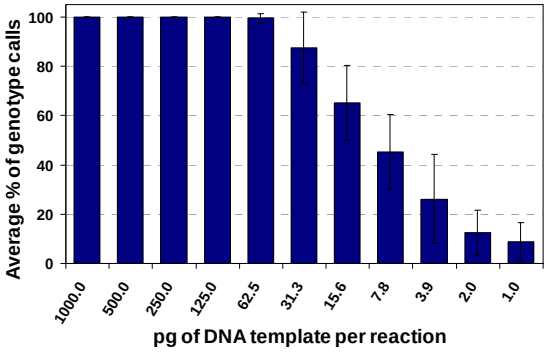


Figure 7. Sensitivity of 8-well Y-STR assay using purified DNA template. Sensitivity was measured with duplicate dilution series of six different templates prepared from male human blood. The average percentage of loci correctly called (out of 16) for each of the 12 dilution series is graphed ± the standard deviation for the 12 runs. At 62.5 pg per reaction, one allele was missed from DYS437 (allele 15) from one replicate of sample 55-25290.

developmental validation of the Y-STR assay has been performed, including sensitivity, species specificity, reproducibility, concordance and precision/accuracy tests, as well as a survey of population samples to assess polymorphism frequencies in core Y-STR loci.

To assess sensitivity, dilution series from 1000 pg per reaction to 1.0 pg per reaction were performed in duplicate on six independent DNA templates that had been purified from blood. Full profiles were detected in 11 of 12 replicates at 62.5 pg/reaction (one allele call was missed in one replicate). Full profiles were produced at 125, 250, 500 and 1000 pg/reaction (Figure 7).

Male DNA has been tested in triplicate in the presence and absence of a 10-fold excess of DNA from six different non-human species. DNA from two vertebrate species (domestic dog and cat), filamentous fungus (*Aspergillus oryzae*), yeast (*Candida albicans*), gram negative bacteria (*Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus*) was used at 10 ng per reaction in the absence and presence of 1 ng of human. Data produced in the presence of exogenous DNA are shown in the main report. No specific products attributable to exogenous DNAs were detected.

A considerable effort was required in assay configuration and primer design to ensure exclusion of cross-priming in the presence of female DNA. A detailed description of steps taken is presented in the main body of the report. First, original primers designed to DYS393 produced products from a homologous locus on the X-chromosome. A primer pair was used that contains a 2-base mismatch to the X-chromosome homolog on the 3′ end of the reverse primer. Second, a primer pair designed against DYS456 produced multiple products that appeared at ≥3 ng of female DNA and could be seen in the presence of 1 ng male DNA when female DNA was ≥25 ng. Third, primer pairs directed against Y-GATA-H4 and DYS439 produced cross products on the X-chromosome when in combination by virtue of the one primer from each pair cooperating in the presence of the X-chromosome (but not the Y-chromosome). These products were likewise visible at ≥3 ng of female DNA and could be seen in the presence of 1 ng male DNA when female DNA was ≥25 ng. These issues have been resolved and with the current assay, 100 ng of female DNA per reaction does not interfere with generation of a profile from 1 ng/reaction of male DNA.

Utilizing data generated with 335 runs of 214 individuals, the distribution of mass measurement deviations from expected was assessed. The data were evaluated for 11,298 individual product strand assignments (5,649 double-stranded allele assignments). The average mass measurement deviation magnitude (absolute value of mass measurement deviations from expected) was 13.0 ± 10.8 ppm (Figure 8), and was comparable to the

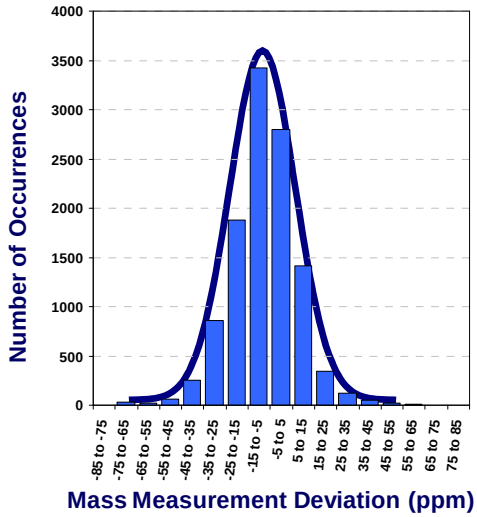


Figure 8. Distribution of mass measurement deviations for a 11,298 mass-product strand assignments produced from 335 runs of 214 male templates.

STR assay.

To assess the ability to generate profiles concordant with existing "Gold standard" technology with the full set of 16 markers used in the final assay, 34 blood-derived male DNA samples were amplified at Ibis with the AB Y-Filer™ system and analyzed on an AB 310 single-capillary instrument in-house. The same 34 samples were then analyzed using a preliminary Ibis kit. All nominal allele calls were concordant, and all deduced DYS389II assignments, which were obtained by simply adding the allele numbers for DYS389I and DYS389II-1, were concordant with DYS389II assignments made with the Y-Filer system.

Specific Aim 3: Characterize polymorphisms in core autosomal STR and Y-STR markers

In collaboration with Art Eisenberg and John Planz at the University at North Texas Health Sciences Center (UNTHSC), we have surveyed polymorphisms in core autosomal STR loci for a set of 847 samples, which, combined with 95 samples from NIST, consists of 297 Caucasian, 332 African American and 313 Hispanic samples. Allele frequencies, including polymorphisms, are shown in Table 5 for these 942 samples.

Table 5, part 1. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
CSF1PO	11	177	154	181	29.8	23.2	28.9
	13	45	38	38	7.6	5.7	6.1
	10	156	193	158	26.3	29.1	25.2
	12	195	170	223	32.8	25.6	35.6
	7	0	41	4	0.0	6.2	0.6
	6	0	1	0	0.0	0.2	0.0
	8	0	38	3	0.0	5.7	0.5
	9	14	25	14	2.4	3.8	2.2
	11S2	0	1	0	0.0	0.2	0.0
	12S2	0	1	0	0.0	0.2	0.0
	14	5	2	4	0.8	0.3	0.6
	15	2	0	1	0.3	0.0	0.2
D13S317	9	36	15	125	6.1	2.3	20.0
	12S9	73	80	63	12.3	12.0	10.1
	11	71	111	68	12.0	16.7	10.9
	12	115	205	94	19.4	30.9	15.0
	8	58	19	53	9.8	2.9	8.5
	11S9	109	88	50	18.4	13.3	8.0
	13	42	84	63	7.1	12.7	10.1
	14	29	18	33	4.9	2.7	5.3
	13S9	17	23	16	2.9	3.5	2.6
	10	31	8	22	5.2	1.2	3.5
	10S9	6	8	36	1.0	1.2	5.8
	14S9	6	3	1	1.0	0.5	0.2
	9S9	0	1	1	0.0	0.2	0.2
	15	0	1	0	0.0	0.2	0.0
	13S4	1	0	0	0.2	0.0	0.0
	15S9	0	0	1	0.0	0.0	0.2
D3S1358	16S1.2	13	113	12	2.2	17.0	1.9
	17S1	63	83	31	10.6	12.5	5.0
	15S1.2	21	108	19	3.5	16.3	3.0
	16S1	88	65	102	14.8	9.8	16.3
	14S1	73	48	65	12.3	7.2	10.4
	17	43	60	51	7.2	9.0	8.1
	16	44	37	33	7.4	5.6	5.3
	18	67	26	54	11.3	3.9	8.6
	15S1	148	61	222	24.9	9.2	35.5
	13S1.2	0	3	0	0.0	0.5	0.0
	12S1.2	0	2	0	0.0	0.3	0.0
	15	6	13	6	1.0	2.0	1.0
	17S1.2	4	19	7	0.7	2.9	1.1
	13S1	1	1	6	0.2	0.2	1.0
	19	6	1	3	1.0	0.2	0.5
	18S1.2	0	4	1	0.0	0.6	0.2
	18S1	8	6	7	1.3	0.9	1.1
	14S1.2	0	10	3	0.0	1.5	0.5
	14	1	1	2	0.2	0.2	0.3
	15.1S1S10	0	1	0	0.0	0.2	0.0
	15.2S1	0	1	0	0.0	0.2	0.0
	17S3	0	1	0	0.0	0.2	0.0
	11S1	3	0	1	0.5	0.0	0.2
	19S1	1	0	0	0.2	0.0	0.0
	16S2	1	0	0	0.2	0.0	0.0
	17S2	3	0	1	0.5	0.0	0.2

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D21S11	29	105	111	101	17.7	16.7	16.1
	31	26	49	37	4.4	7.4	5.9
	31.2	55	34	59	9.3	5.1	9.4
	28	86	157	65	14.5	23.6	10.4
	30S2	69	94	127	11.6	14.2	20.3
	27S2	18	16	7	3.0	2.4	1.1
	33.2	15	21	23	2.5	3.2	3.7
	37S2.2	0	1	0	0.0	0.2	0.0
	32.2	63	45	82	10.6	6.8	13.1
	27	1	15	2	0.2	2.3	0.3
	35S2.2	0	3	1	0.0	0.5	0.2
	29S1	29	6	18	4.9	0.9	2.9
	29S2	1	13	0	0.2	2.0	0.0
	30S2.2	0	3	1	0.0	0.5	0.2
	30	72	23	46	12.1	3.5	7.3
	32S2	2	14	4	0.3	2.1	0.6
	30.2	16	7	8	2.7	1.1	1.3
	35.2	3	2	0	0.5	0.3	0.0
	31S1	14	7	9	2.4	1.1	1.4
	32	4	3	2	0.7	0.5	0.3
	25.2	1	1	0	0.2	0.2	0.0
	33S2	0	1	0	0.0	0.2	0.0
	35S7	0	10	0	0.0	1.5	0.0
	36S2.2	0	2	0	0.0	0.3	0.0
	34.2	0	4	2	0.0	0.6	0.3
	34S2.2	0	1	0	0.0	0.2	0.0
	35	1	4	0	0.2	0.6	0.0
	33.1	0	2	1	0.0	0.3	0.2
	33	0	2	1	0.0	0.3	0.2
	29.2S2	2	1	0	0.3	0.2	0.0
	33.2S2	0	1	1	0.0	0.2	0.2
	34	0	2	1	0.0	0.3	0.2
	34S2S11	0	1	0	0.0	0.2	0.0
	31.2S1	2	1	5	0.3	0.2	0.8
	26	0	1	2	0.0	0.2	0.3
	36	0	1	0	0.0	0.2	0.0
	36S2	0	1	0	0.0	0.2	0.0
	28S1	1	1	1	0.2	0.2	0.2
	36S2S10	0	1	0	0.0	0.2	0.0
	37S2.3S1	0	1	0	0.0	0.2	0.0
	37S2.3	0	1	0	0.0	0.2	0.0
	29.3	1	0	0	0.2	0.0	0.0
	32.2S1	1	0	9	0.2	0.0	1.4
	30.2S1	4	0	3	0.7	0.0	0.5
	33S1	1	0	2	0.2	0.0	0.3
	29.2	1	0	0	0.2	0.0	0.0
	30S2S11	0	0	2	0.0	0.0	0.3
	33.2S1	0	0	3	0.0	0.0	0.5
	28.2	0	0	1	0.0	0.0	0.2
AMEL	X	428	459	463	72.1	69.1	74.0
	Y	166	205	163	27.9	30.9	26.0

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

Table 5, part 2. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D18S51	15	87	109	112	14.6	16.4	17.9
	19	30	54	16	5.1	8.1	2.6
	17	70	105	93	11.8	15.8	14.9
	18	38	66	41	6.4	9.9	6.5
	10	8	3	0	1.3	0.5	0.0
	16	75	117	60	12.6	17.6	9.6
	20	12	26	13	2.0	3.9	2.1
	24	1	3	2	0.2	0.5	0.3
	12	88	54	69	14.8	8.1	11.0
	14.2S4	0	2	0	0.0	0.3	0.0
	15.2	0	2	0	0.0	0.3	0.0
	22	2	7	10	0.3	1.1	1.6
	21	3	9	6	0.5	1.4	1.0
	14	87	47	106	14.6	7.1	16.9
	13	80	34	74	13.5	5.1	11.8
	9	0	2	0	0.0	0.3	0.0
	17S4	0	1	2	0.0	0.2	0.3
	16S4	0	1	0	0.0	0.2	0.0
	14S7	3	4	4	0.5	0.6	0.6
	18S4	0	2	1	0.0	0.3	0.2
	21.2	0	1	0	0.0	0.2	0.0
	12S4	0	2	0	0.0	0.3	0.0
	11	8	2	9	1.3	0.3	1.4
	13S4	0	1	0	0.0	0.2	0.0
	19S4	0	1	0	0.0	0.2	0.0
	13.2S4	1	3	0	0.2	0.5	0.0
	11S4	0	1	0	0.0	0.2	0.0
	20S4	0	4	0	0.0	0.6	0.0
	23	0	1	3	0.0	0.2	0.5
	15S7	1	0	1	0.2	0.0	0.2
	25	0	0	3	0.0	0.0	0.5
	15S4.2	0	0	1	0.0	0.0	0.2
D7S820	10	130	191	144	21.9	28.8	23.0
	9	76	101	55	12.8	15.2	8.8
	11	113	119	174	19.0	17.9	27.8
	13	14	9	20	2.4	1.4	3.2
	12	70	60	86	11.8	9.0	13.7
	8	98	150	71	16.5	22.6	11.3
	12S10	21	11	23	3.5	1.7	3.7
	14	6	3	2	1.0	0.5	0.3
	11S10	13	3	16	2.2	0.5	2.6
	10S10	32	8	20	5.4	1.2	3.2
	7	12	6	9	2.0	0.9	1.4
	13S10	5	2	2	0.8	0.3	0.3
	11S2	0	1	0	0.0	0.2	0.0
	9S4	2	0	0	0.3	0.0	0.0
	9S10	1	0	1	0.2	0.0	0.2
	7S10	1	0	0	0.2	0.0	0.0
	10.3	0	0	3	0.0	0.0	0.5

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D5S818	12	163	182	128	27.4	27.4	20.4
	13	56	120	61	9.4	18.1	9.7
	11	205	126	272	34.5	19.0	43.5
	12S8	65	62	31	10.9	9.3	5.0
	8S8	0	50	3	0.0	7.5	0.5
	11S8	24	24	16	4.0	3.6	2.6
	13S8	21	34	10	3.5	5.1	1.6
	9	0	1	0	0.0	0.2	0.0
	10S8	6	4	9	1.0	0.6	1.4
	9S8	20	12	31	3.4	1.8	5.0
	10	20	24	21	3.4	3.6	3.4
	14	6	10	6	1.0	1.5	1.0
	13S6	1	8	3	0.2	1.2	0.5
	15S6	0	1	0	0.0	0.2	0.0
	7	1	1	33	0.2	0.2	5.3
	14S6	0	2	0	0.0	0.3	0.0
	14S8	2	2	1	0.3	0.3	0.2
	15	2	1	1	0.3	0.2	0.2
	8	2	0	0	0.3	0.0	0.0
D8S1179	11	44	22	38	7.4	3.3	6.1
	14S2	96	219	138	16.2	33.0	22.0
	13S2	160	120	137	26.9	18.1	21.9
	16S2.2	0	10	0	0.0	1.5	0.0
	12S2	4	38	13	0.7	5.7	2.1
	15S2	53	107	60	8.9	16.1	9.6
	10	55	8	68	9.3	1.2	10.9
	12	91	33	66	15.3	5.0	10.5
	15S2.2	0	18	0	0.0	2.7	0.0
	17S2	2	3	1	0.3	0.5	0.2
	13	42	26	52	7.1	3.9	8.3
	16S2	15	34	13	2.5	5.1	2.1
	14	15	8	23	2.5	1.2	3.7
	11S2	0	6	1	0.0	0.9	0.2
	17S2.2	0	4	0	0.0	0.6	0.0
	8	7	4	4	1.2	0.6	0.6
	17S2.3	0	1	0	0.0	0.2	0.0
	9	7	2	2	1.2	0.3	0.3
	13S2.2	0	1	0	0.0	0.2	0.0
	14S2S5	2	0	0	0.3	0.0	0.0
	14S2.2S12	1	0	0	0.2	0.0	0.0
	13S2S5	0	0	6	0.0	0.0	1.0
	16	0	0	1	0.0	0.0	0.2
	15	0	0	2	0.0	0.0	0.3
	18S2	0	0	1	0.0	0.0	0.2
TPOX	8	308	234	341	51.9	35.2	54.5
	9	62	134	43	10.4	20.2	6.9
	11	143	126	142	24.1	19.0	22.7
	7	1	14	3	0.2	2.1	0.5
	10	52	78	25	8.8	11.7	4.0
	12	27	15	70	4.5	2.3	11.2
	6	1	62	1	0.2	9.3	0.2
	13	0	1	1	0.0	0.2	0.2

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

Table 5, part 3. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
vWA	17	139	107	151	23.4	16.1	24.1
	18	118	88	102	19.9	13.3	16.3
	15S1	59	32	37	9.9	4.8	5.9
	20	5	6	6	0.8	0.9	1.0
	15	10	96	33	1.7	14.5	5.3
	17S1	10	28	12	1.7	4.2	1.9
	18S1	4	11	2	0.7	1.7	0.3
	20S2.2	0	1	0	0.0	0.2	0.0
	16	93	116	187	15.7	17.5	29.9
	16S1	21	56	14	3.5	8.4	2.2
	14S2S4.2	49	23	32	8.2	3.5	5.1
	14S1S4	16	6	5	2.7	0.9	0.8
	14S4	3	21	3	0.5	3.2	0.5
	19S2.2	0	7	0	0.0	1.1	0.0
	20S2	1	6	0	0.2	0.9	0.0
	13S3	1	10	0	0.2	1.5	0.0
	19	52	27	35	8.8	4.1	5.6
	18.3	1	1	0	0.2	0.2	0.0
	19S2	1	2	2	0.2	0.3	0.3
	18S2.2	0	2	0	0.0	0.3	0.0
	17S2S11	1	1	0	0.2	0.2	0.0
	19S1	1	3	0	0.2	0.5	0.0
	18S2	2	4	1	0.3	0.6	0.2
	20S2.3	0	1	0	0.0	0.2	0.0
	11	0	5	0	0.0	0.8	0.0
	21S2	0	1	1	0.0	0.2	0.2
	12	0	1	0	0.0	0.2	0.0
	13S1S3	1	1	0	0.2	0.2	0.0
	21S2S2	0	1	0	0.0	0.2	0.0
	17S2	1	0	0	0.2	0.0	0.0
	21	1	0	0	0.2	0.0	0.0
	18S10	2	0	1	0.3	0.0	0.2
	15S2S4.2	1	0	1	0.2	0.0	0.2
	20S10	1	0	0	0.2	0.0	0.0
	18S2.2S11	0	0	1	0.0	0.0	0.2
D16S539	9	68	154	58	11.4	23.2	9.3
	12	178	119	174	30.0	17.9	27.8
	11	179	185	187	30.1	27.9	29.9
	14	17	11	10	2.9	1.7	1.6
	13	108	94	88	18.2	14.2	14.1
	10	33	76	103	5.6	11.4	16.5
	8	10	23	3	1.7	3.5	0.5
	7	0	1	0	0.0	0.2	0.0
	10S9.2	0	1	0	0.0	0.2	0.0
	9S2	1	0	0	0.2	0.0	0.0
	8S5	0	0	3	0.0	0.0	0.5

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
FGA	23	81	119	83	13.6	17.9	13.3
	31.2	1	5	0	0.2	0.8	0.0
	24	85	106	89	14.3	16.0	14.2
	21	114	67	88	19.2	10.1	14.1
	30	0	2	0	0.0	0.3	0.0
	20	68	47	52	11.4	7.1	8.3
	22	110	119	90	18.5	17.9	14.4
	25	50	79	93	8.4	11.9	14.9
	43.2	0	1	0	0.0	0.2	0.0
	19	34	43	45	5.7	6.5	7.2
	44.2S7.2	0	1	0	0.0	0.2	0.0
	26	16	19	47	2.7	2.9	7.5
	28S4	0	6	1	0.0	0.9	0.2
	24S7	0	1	0	0.0	0.2	0.0
	27S4	0	12	0	0.0	1.8	0.0
	18.2	0	8	0	0.0	1.2	0.0
	18	13	4	6	2.2	0.6	1.0
	44.2S7.2S4	0	1	0	0.0	0.2	0.0
	19.2	0	4	0	0.0	0.6	0.0
	44.2S7S4	0	1	0	0.0	0.2	0.0
	16.1	0	1	0	0.0	0.2	0.0
	20.2	0	2	0	0.0	0.3	0.0
	46.2S6.2S8	0	1	0	0.0	0.2	0.0
	29	0	2	1	0.0	0.3	0.2
	24S4	0	1	0	0.0	0.2	0.0
	28S4.2	0	1	0	0.0	0.2	0.0
	47.2S8.2S6	0	1	0	0.0	0.2	0.0
	26S4	1	4	0	0.2	0.6	0.0
	32.2	0	1	0	0.0	0.2	0.0
	17	0	1	0	0.0	0.2	0.0
	24.2	0	1	1	0.0	0.2	0.2
	17.2	0	1	0	0.0	0.2	0.0
	30.2	0	1	0	0.0	0.2	0.0
	23.2	5	1	0	0.8	0.2	0.0
	22.2	7	0	2	1.2	0.0	0.3
	25S12	2	0	1	0.3	0.0	0.2
	27	4	0	12	0.7	0.0	1.9
	21.2	2	0	3	0.3	0.0	0.5
	26S5	1	0	0	0.2	0.0	0.0
	28	0	0	5	0.0	0.0	0.8
	23S5	0	0	3	0.0	0.0	0.5
	25.3	0	0	1	0.0	0.0	0.2
	15	0	0	1	0.0	0.0	0.2
	25.2S4	0	0	1	0.0	0.0	0.2
	23.2S4	0	0	1	0.0	0.0	0.2
THO1	9	82	95	72	13.8	14.3	11.5
	7	123	293	230	20.7	44.1	36.7
	9.3	191	62	123	32.2	9.3	19.6
	8	55	126	44	9.3	19.0	7.0
	6	138	86	155	23.2	13.0	24.8
	10	2	2	2	0.3	0.3	0.3
	5	3	0	0	0.5	0.0	0.0

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

In addition to 95 samples from NIST run in a preliminary 11-locus Y-STR assay, 187 samples obtained from John Planz at UNTHSC comprising 74 African American, 58 Caucasian and 45 Hispanic samples were run in the 16-locus Y-STR assay. Although at least one SNP was observed in 12 of 16 loci, only three loci appeared to have a substantial number of polymorphic alleles. Each of these also appeared to present a level of population bias in SNP frequency for the three populations surveyed (Figure 9).

Locus	Caucasian SNP %	African American SNP %	Hispanic SNP %
DYS385a/b	0.0	0.9	1.7
DYS389II-1	5.6	67.3	17.0
DYS390	6.7	4.7	6.9
DYS392	1.1	0.9	0.0
DYS393	3.4	0.0	1.1
DYS437	1.1	59.4	4.6
DYS438	1.1	0.0	3.4
DYS439	0.0	0.0	1.1
DYS448	0.0	1.4	0.0
DYS458	6.9	1.4	5.5
DYS635	37.3	73.0	29.6
Y-GATA-H4	0.0	1.4	0.0

187 samples with 16-locus assay

- 74 African American
- 58 Caucasian
- 45 Hispanic

95 samples with preliminary 11-locus assay

- 32 African American
- 31 Caucasian
- 32 Hispanic

Figure 9. Frequency of SNPs observed in Y-STR loci for 187 samples surveyed at 16 loci and 95 samples surveyed at 11 loci.

Specific Aim 4: Analysis of extended family samples

A panel of samples received from UNTHSC containing groups of two parents plus one or more offspring where the sample set is known to contain parent/offspring combinations having parent-to-offspring STR mutations (e.g., an allele 12 from a parent becomes an allele 11 in the offspring) were tested in the Ibis STR system. The samples came blinded without information about the mutations in question or the parent-offspring relationships (other than code numbers that indicated which parents and offspring belonged together). The samples were genotyped using a scaled-down panel of primer pairs containing only the primer pairs known to contain a high frequency of SNP polymorphisms, namely D13S317, D21S11, D3S1358, D5S818, D7S820, D8S1179 and vWA. The loci were surveyed in custom plates containing two triplex reactions and one single-plex (D21S11), allowing 32 samples per 96-well plate to be analyzed (30 samples plus one positive and one negative control).

Profiles for the seven most polymorphic loci have been registered for family groupings containing a mother, a presumed father, and one or more offspring (Table 6). Samples were grouped into 80 family groupings where one offspring had a demonstrated germline mutation in an allele from one of the seven loci surveyed. Data shown in Table 6 demonstrate that polymorphisms observed in STR alleles are faithfully transmitted from parent to offspring and are not an artifact of the methodology used to assay them. We have not yet seen a demonstrable case of a two parent-offspring trio that suggests that an allele from a parent gained or lost a SNP polymorphism between a parent and a child.

There are some interesting consequences of the increased discrimination of alleles afforded by the ability to detect polymorphisms within STR alleles when dealing with samples from related individuals. For example, if one examines the D5S818 genotypes that would results from standard typing for the three individuals of group 3, the genotypes would be: mother [11, 13], father [11, 13], child [12, 13]. It would therefore be considered possible that the mother contributed allele 13 and that the father contributed either an 11 or 13 that mutated to a 12, or alternatively that the father

contributed allele 13 and that the mother contributed either an 11 or 13 that mutated to a 12. There would therefore be four distinct scenarios that could lead to the child's genotype. The mass spectrometry-based assay, however, produced the genotypes mother [11, 13], father [11, 13 (G→T)], child [12 (G→T), 13]. It is now straightforward to see that there is only one viable explanation for the path of mutation. The father's allele 13 (G→T) presumably mutated to a 12 (G→T) through replication slippage).

Another interesting case is group 61, sample UNTHSC0034-M0363C2, locus D8S1179. In this case, with conventional typing, the mother's genotype would be [14, 14], the father's would be [14, 15] and the child's would be [13, 14]. It would therefore be possible that, provided that these are the true parents (it could be imagined that this could be a paternity case) the allele 13 could have come from the allele 14 of either the (known) mother or (assumed) father. With the mass spectrometry-based assay, the genotypes are mother [14, 14 (A→G)], father [14 (A→G), 15 (A→G)], and child [13, 14 (A→G)]. It is most plausible that the father actually contributed the allele 14 (A→G), requiring no hypothesis of a mutation in the father's germline, and the mother contributed her allele 14 that mutated to a 13 in the child. Figure 10 shows the data for these D8S1179 genotypes.

Table 6. Seven-locus profiles members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Locus	Change
1	UNTHSC0031-M0188A1	9, 12	30 (A->G), 31	17, 18	11, 11	10, 12 (T->A)	13, 13 (A->G)	16, 16		
1	UNTHSC0031-M0188B1	12, 12	29, 31.2	18, 18	11, 12 (G->T)	8, 12	13, 13 (A->G)	18, 19		
1	UNTHSC0031-M0188C1	12, 12	31, 31.2	17, 18	11, 11	10, 12	13, 13	16, 20	vWA	19->20
2	UNTHSC0031-M0033A1	11 (A->T), 12 (A->T)	31, 31	16 (G->A), 17	12, 14	10, 10	13 (A->G), 14 (A->G)	17, 18		
2	UNTHSC0031-M0033B1	11, 13 (A->T)	29 (G->A), 34.2	15 (2G->2A), 17	12, 13	8, 8	8, 14 (A->G)	16, 19		
2	UNTHSC0031-M0033C1	12 (A->T), 13 (A->T)	29 (G->A), 30 (A->G)	15 (2G->2A), 16 (G->A)	12, 12	8, 10	14 (A->G), 14 (A->G)	16, 17	D21S11	31->30
3	UNTHSC0031-M0096A1	12, 12	29, 30 (A->G)	16 (2G->2A), 16 (2G->2A)	11, 13	9, 11	13 (A->G), 13 (A->G)	16, 18		
3	UNTHSC0031-M0096B1	11, 12	27, 32.2	15 (G->A), 17	11, 13 (G->T)	10, 11 (T->A)	12 (A->G), 16 (A->G)	16 (G->A), 18		
3	UNTHSC0031-M0096C1	11, 12	29, 32.2	16 (2G->2A), 17	12 (G->T), 13	11, 11 (T->A)	13 (A->G), 16 (A->G)	16, 18	D5S818	13 (G->T)-> 12(G->T)
4	UNTHSC0031-M0073A1	9, 11	28, 31.2	15 (G->A), 16 (G->A)	11, 11	12, 12 (T->A)	10, 14 (A->G)	14 (A->G + 2T->2C), 19		
4	UNTHSC0031-M0073B1	12, 12	29 (G->A), 31 (G->A)	15 (G->A), 18	9 (G->T), 13	8, 13 (T->A)	10, 12	16, 17		
4	UNTHSC0031-M0073C1	11, 12	31 (G->A), 31.2	15 (G->A), 16 (G->A)	11, 13	12 (T->A), 12 (T->A)	12, 14 (A->G)	14 (A->G + 2T->2C), 16	D7S820	13 (T->A)-> 12 (T->A)
5	UNTHSC0031-M0072A1	12 (A->T), 13 (A->T)	28, 31	14 (G->A), 17	11, 11	11, 12	13, 14	14 (A->G + 2T->2C), 16		
5	UNTHSC0031-M0072B1	8, 9	30.2 (G->A), 31 (G->A)	15 (G->A), 16	11, 13	10, 12	10, 10	14 (A->G + 2T->2C), 15 (G->A)		
5	UNTHSC0031-M0072C1	8, 13 (A->T)	30.2 (G->A), 31	14 (G->A), 15 (G->A)	11, 11	11, 11	10, 13	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	D7S820	12->11 or 10->11
6	UNTHSC0031-M0143A1	8, 12	30 (A->G), 31 (G->A)	15 (G->A), 15 (G->A)	11 (G->T), 13 (G->T)	8, 10	13 (A->G), 15 (A->G)	17, 17		
6	UNTHSC0031-M0143B1	8, 13	29, 30 (A->G)	16 (2G->2A), 17 (G->A)	12, 12	8, 15	12, 14	17, 18		
6	UNTHSC0031-M0143C1	12, 13	30 (A->G), 31 (G->A)	15 (G->A), 16 (2G->2A)	11 (G->T), 12	8, 10	13, 15 (A->G)	17, 17	D8S1179	14->13 or 12->13
7	UNTHSC0031-M0296A1	12, 14	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 12 (G->T)	8, 8	12, 14 (A->G)	16, 20		
7	UNTHSC0031-M0296B1	12, 13	29, 32.2	16 (2G->2A), 16 (2G->2A)	11, 14 (G->T)	11, 11	14 (A->G), 16 (2A->2G)	17, 18		
7	UNTHSC0031-M0296C1	12, 14	30 (A->G), 32.2	15 (G->A), 16 (2G->2A)	11, 11	8, 11	14 (A->G), 17 (2A->2G)	18, 20	D8S1179	16 (2A->2G)-> 17 (2A->2G)
8	UNTHSC0031-M0292A1	12, 12 (A->T)	31.2 (G->A), 32.2	15 (G->A), 15 (G->A)	7, 12	11, 12	13, 13	17, 18		
8	UNTHSC0031-M0292B1	12, 12 (A->T)	29, 29	15 (G->A), 18 (G->C)	11, 12	10, 11	10, 14 (A->G)	14 (A->G + 2T->2C), 17		
8	UNTHSC0031-M0292C1	12, 12 (A->T)	29, 31.2 (G->A)	15 (G->A), 18 (G->C)	7, 12	11, 11	13, 14 (A->G)	17, 18		
9	UNTHSC0031-M0294A1	8, 11 (A->T)	29 (G->A), 30 (A->G)	15 (2G->2A), 16 (G->A)	10 (G->T), 11	10, 11	13, 13 (A->G)	17, 18		
9	UNTHSC0031-M0294B1	10 (A->T), 11	30, 30.2 (G->A)	16 (G->A), 17	12, 12	11, 11	12 (A->G), 13	17, 18		
9	UNTHSC0031-M0294C1	10 (A->T), 11 (A->T)	29 (G->A), 30	15 (2G->2A), 17	11, 12	11, 11	12 (A->G), 13	17, 17		
10	UNTHSC0031-M0293A1	12, 12 (A->T)	29, 31	15 (G->A), 16	11, 11	8, 11 (T->A)	13 (A->G), 14 (A->G)	16, 20		
10	UNTHSC0031-M0293B1	10, 11 (A->T)	28, 29 (G->A)	15 (G->A), 16 (G->A)	11, 12	11, 11	13 (A->G), 13 (A->G)	16, 17		
10	UNTHSC0031-M0293C1	11 (A->T), 12 (A->T)	29 (G->A), 31	15 (G->A), 15 (G->A)	11, 12	8, 11	13 (A->G), 13 (A->G)	16, 16		
11	UNTHSC0031-M0295A1	11, 11 (A->T)	30 (A->G), 30 (A->G)	16 (G->A), 18	10, 13 (G->C)	10, 12 (T->A)	10, 14 (A->G)	16, 19		
11	UNTHSC0031-M0295B1	11 (A->T), 12	27, 29	15 (G->A), 16 (2G->2A)	12 (G->T), 14	10, 12	13, 16 (A->G)	15, 16		
11	UNTHSC0031-M0295C1	11 (A->T), 11 (A->T)	27, 30 (A->G)	16 (2G->2A), 16 (G->A)	10, 12 (G->T)	10, 12	13, 14 (A->G)	16, 16		
12	UNTHSC0031-M0099A1	12, 13	31, 33.2	15 (G->A), 17 (G->A)	11, 12	11, 12	13, 14 (A->G)	15, 16 (G->A)		
12	UNTHSC0031-M0099B1	8, 9	25 (3A->3G), 30 (A->G)	15 (G->A), 18	12, 14 (G->T)	11, 12	12, 15 (A->G)	15 (G->A), 17		
12	UNTHSC0031-M0099C1	8, 13	25 (3A->3G), 31	15 (G->A), 18	11, 13 (G->T)	11, 11	13, 15 (A->G)	15, 15 (G->A)	D5S818	14 (G->T)-> 13 (G->T)
13	UNTHSC0032-M0311A1	11 (A->T), 12 (A->T)	29, 29	15 (2G->2A), 16 (2G->2A)	13, 13 (G->T)	8, 10	12, 12 (A->G)	15 (G->A), 16		
13	UNTHSC0032-M0311B1	12, 13 (A->T)	29 (A->G), 31	17 (G->A), 18	11, 12 (G->T)	9, 10	13 (A->G), 14 (A->G)	11, 14 (A->G + 2T->2C)		
13	UNTHSC0032-M0311C1	12 (A->T), 13 (A->T)	29, 32 (A->G)	15 (2G->2A), 17 (G->A)	11, 13	8, 9	12, 14 (A->G)	14 (A->G + 2T->2C), 15 (G->A)	D21S11	31->32
14	UNTHSC0032-M0313A1	12, 12 (A->T)	29, 30 (A->G)	15 (2G->2A), 16	12, 13 (G->C)	11, 12	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17 (G->A)		
14	UNTHSC0032-M0313B1	13, 13 (A->T)	28, 32.2	14 (G->A), 19 (G->A)	10, 12 (G->T)	8, 12	15 (2A->2G), 15 (A->G)	15 (G->A), 18 (G->A)		
14	UNTHSC0032-M0313C1	12 (A->T), 13 (A->T)	29, 32.2	14 (G->A), 16	12 (G->T), 13 (G->C)	8, 12	13 (A->G), 15 (2A->2G)	17 (G->A), 17 (G->A)	vWA	18 (G->A)->17 (G >A)
15	UNTHSC0032-M0315A1	10, 12	31.2, 32.2	14 (G->A), 18	11, 14	8, 11	10, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
15	UNTHSC0032-M0315B1	8, 9	32.2, 33.2	16 (2G->2A), 18	11, 13	10, 12	10, 13 (A->G)	15 (G->A), 16		
15	UNTHSC0032-M0315C1	8, 12	31.2, 32.2	14 (G->A), 18	13, 13	11, 12	10, 10	14 (A->G + 2T->2C), 15 (G->A)	D5S818	14->13
16	UNTHSC0032-M0316A1	11 (A->T), 12	30.2 (G->A), 31.2	16, 18	11, 12	10, 10 (T->A)	11, 12	16 (G->A), 19		
16	UNTHSC0032-M0316B1	10, 13	29, 34.2	14 (G->A), 15 (G->A)	12 (G->T), 13	8, 12	9, 13 (A->G)	15 (G->A), 17		
16	UNTHSC0032-M0316C1	11 (A->T), 12	29, 31.2	15 (G->A), 16	11, 13	10, 12	9, 12	15 (G->A), 16 (G->A)	D13S317	13->12
17	UNTHSC0032-M0317A1	11, 14	27 (A->G), 28	15 (G->A), 16 (G->A)	12 (G->T), 13	10, 11	13 (A->G), 14 (A->G)	15, 20 (A->G)		
17	UNTHSC0032-M0317C1	12, 14	28, 28	14 (G->A), 15 (G->A)	12, 13	11, 11	13 (A->G), 13 (A->G)	18, 20 (A->G)	vWA	19->18
17	UNTHSC0036-M0317B1	11, 12	28, 32 (A->G)	14 (G->A), 16 (G->A)	12, 12	10, 11	13 (A->G), 14 (A->G)	16, 19		
18	UNTHSC0032-M0318B1	12, 12	30, 32.2	16 (G->A), 16 (G->A)	11, 12	10, 11	10, 10	17, 20		
18	UNTHSC0032-M0318C1	10 (A->T), 12	30, 31	15 (G->A), 16 (G->A)	11, 11	10, 11	10, 10	17, 17	D21S11	30->31
18	UNTHSC0036-M0318A1	10 (A->T), 11 (A->T)	30 (A->G), 32 (A->G)	15 (G->A), 17	11, 11	11, 11	10, 12	16, 17		
19	UNTHSC0032-M0319A1	12 (A->T), 13	30 (A->G), 32.2	16 (G->A), 16 (G->A)	12 (G->T), 13	11, 11	10, 15 (A->G)	17, 18		
19	UNTHSC0032-M0319B1	9, 12 (A->T)	28, 31.2	16 (G->A), 16 (G->A)	9 (G->T), 12	9, 11	10, 11	17, 18		
19	UNTHSC0032-M0319C1	12 (A->T), 13	31.2, 32.2	16 (G->A), 16 (G->A)	9 (G->T), 12 (G->T)	11, 11	10, 15 (A->G)	16, 17	vWA	17->16
20	UNTHSC0032-M0320A1	12, 14	27 (A->G), 30 (A->G)	17 (G->A), 17 (G->A)	12, 13 (G->C)	10, 13	12 (A->G), 17 (2A->2G)	16, 16 (G->A)		
20	UNTHSC0032-M0320B1	11 (A->T), 12	28, 30.2	15, 15 (2G->2A)	9 (G->T), 12 (G->T)	8, 11	13 (A->G), 14 (A->G)	15 (G->A), 17		
20	UNTHSC0032-M0320C1	12, 14	27 (A->G), 28	16, 17 (G->A)	9 (G->T), 13 (G->C)	8, 13	12 (A->G), 14 (A->G)	15 (G->A), 16	D3S1358	15->16
21	UNTHSC0032-M0321A1	9, 12	30, 30 (A->G)	15 (G->A), 15 (G->A)	12 (G->T), 13	10, 11	13 (A->G), 13 (A->G)	16, 16		
21	UNTHSC0032-M0321B1	11, 11 (A->T)	27 (A->G), 30 (A->G)	16, 16 (2G->2A)	11, 13	8, 11	14 (A->G), 15 (A->G)	13 (C->T), 17		
21	UNTHSC0032-M0321C1	9, 11	30, 30 (A->G)	15 (2G->2A), 15 (G->A)	13, 13	10, 11	13 (A->G), 15 (A->G)	13 (C->T), 16	D3S1358	16 (2G->2A)-> 15(2G->2A)
21	UNTHSC0032-M0321C2	9, 11	27 (A->G), 30 (A->G)	15 (G->A), 16 (2G->2A)	11, 13	10, 11	13 (A->G), 14 (A->G)	13 (C->T), 16		
21	UNTHSC0032-M0321C3	9, 11 (A->T)	27 (A->G), 30	15 (G->A), 16 (2G->2A)	11, 12 (G->T)	11, 11	13 (A->G), 15 (A->G)	16, 17		
22	UNTHSC0032-M0322A1	10, 13	30 (A->G), 32.2	18, 18	9 (G->T), 11	9, 11	13 (A->G), 13 (A->G)	16, 17		
22	UNTHSC0032-M0322B1	9, 10	30, 31	18, 18	13, 14	10, 11	14 (A->G), 15 (A->G)	16, 17		
22	UNTHSC0032-M0322C1	9, 10	30 (A->G), 31	18, 18	9 (G->T), 14	11, 11	13 (A->G), 16 (A->G)	17, 17	D8S1179	15 (A->G)-> 16 (A->G)
23	UNTHSC0032-M0323A1	11, 12	30, 32.2	16 (G->A), 18	12, 13 (G->T)	11, 11	13, 13 (A->G)	15 (G->A), 16		
23	UNTHSC0032-M0323B1	11, 12 (A->T)	30, 32.2	15 (G->A), 16	10 (G->T), 12	9, 11	13, 15 (A->G)	17, 18		
23	UNTHSC0032-M0323C1	11, 12	32.2, 32.2	16, 18	10 (G->T), 12	11, 11	12, 13	16, 17	D8S1179	13->12
24	UNTHSC0032-M0325B1	12, 12	27 (A->G), 29.2 (A->G)	15 (G->A), 18	11, 12	10 (T->A), 12	10, 13	18, 18		
24	UNTHSC0032-M0325C1	12, 12 (A->T)	29.2 (A->G), 33.2	16 (G->A), 18	11, 11	10, 12	11, 14	15 (G->A), 18	D8S1179	13->14
24	UNTHSC0036-M0325A1	12, 12 (A->T)	29 (G->A), 33.2	16 (2G->2A), 16 (G->A)	11, 11	10, 14	11, 11	15 (G->A), 18 (G->A)		
25	UNTHSC0032-M0328A1	12, 12	28, 29	16 (2G->2A), 16 (G->A)	11, 11	10, 10	12, 15 (A->G)	15, 19 (2A->2G)		
25	UNTHSC0032-M0328B1	11, 11 (A->T)	28, 31	14 (G->A), 17	12 (G->T), 13	9, 11 (T->A)	13, 14 (A->G)	15, 20 (A->G)		
25	UNTHSC0032-M0328C1	11 (A->T), 12	28, 29	16 (2G->2A), 17	11, 13	10, 10 (T->A)	13, 15 (A->G)	15, 20 (A->G)	D7S820	11 (T->A)-> 10 (T->A)
26	UNTHSC0032-M0300A1	8, 10 (A->T)	29, 32 (A->G)	15 (G->A), 16 (G->A)	12, 12 (G->T)	11, 11 (T->A)	13 (A->G), 14 (A->G)	19, 20		
26	UNTHSC0032-M0300B1	9, 11	28, 30 (A->G)	15 (G->A), 16 (G->A)	9 (G->T), 12	9, 10	10, 13 (A->G)	17, 17		
26	UNTHSC0036-M0300C1	8, 11	30 (A->G), 32 (A->G)	15 (G->A), 16 (G->A)	12, 12 (G->T)	9, 11	13 (A->G), 14 (A->G)	16, 20	vWA	17->16
27	UNTHSC0032-M0301A1	8, 12	31.2, 32.2	15, 18	10, 11	10, 12	13 (A->G), 13 (A->G)	15 (G->A), 16 (G->A)		
27	UNTHSC0032-M0301B1	11, 14	29, 33.2	14 (2G->2A), 18 (G->A)	10, 10	10, 10	14 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 17 (G->A)		
27	UNTHSC0032-M0301C1	12, 14	29, 31.2	14 (2G->2A), 15	11, 11	10, 10	13 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 15 (G->A)	D5S818	10->11
28	UNTHSC0032-M0302A1	10 (A->T), 11	28, 30 (A->G)	14 (G->A), 17	10, 12	12, 12 (T->A)	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 16		
28	UNTHSC0032-M0302B1	12, 12	30 (A->G), 31.2	14 (G->A), 16 (G->A)	11, 12	12, 12	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 17		
28	UNTHSC0032-M0302C1	11, 12	30 (A->G), 30 (A->G)	14 (G->A), 14 (G->A)	10, 12	11, 12	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	D7S820	12->11
29	UNTHSC0032-M0306A1	9, 12	30 (A->G), 32.2	15 (G->A), 17	11, 12	9, 13	10, 14 (A->G)	17, 21		
29	UNTHSC0032-M0306B1	9, 11 (A->T)	29 (G->A), 31	16, 18	10 (G->T), 12 (G->T)	9, 10 (T->A)	14 (A->G), 16 (A->G)	15 (G->A), 18		
29	UNTHSC0032-M0306C1	9, 9	30 (A->G), 31	15 (G->A), 18	12, 12 (G->T)	9, 13	10, 14 (A->G)	18, 21		
30	UNTHSC0032-M0307A1	11 (A->T), 12	30 (A->G), 32.2	15 (G->A), 15 (G->A)	11, 12	10, 11	10, 13 (A->G)	16, 18		
30	UNTHSC0032-M0307C1	11 (A->T), 12 (A->T)	30, 32.2	15 (G->A), 16 (G->A)	11, 11	11, 11	10, 14 (A->G)	17 (G->A), 18	D8S1179	15 (A->G)-> 14 (A->G) or 13 (A->G)-> 14 (A->G)
30	UNTHSC0036-M0307B1	12 (A->T), 13	30, 31 (G->A)	16 (G->A), 16 (G->A)	11, 13	10, 11	13 (A->G), 15 (A->G)	17 (G->A), 18		
31	UNTHSC0032-M0308A1	12, 12	29 (G->A), 32.2	17, 17 (G->A)	11, 12	7, 12 (T->A)	13 (A->G), 14	14 (A->G + 2T->2C), 18		
31	UNTHSC0032-M0308B1	8, 13	32.2, 33.2	16, 16 (G->A)	12, 12	10, 10	13 (A->G), 15 (A->G)	16, 17		
31	UNTHSC0032-M0308C1	12, 13	29 (G->A), 33.2	16, 17	12, 12	10, 12 (T->A)	13 (A->G), 16 (A->G)	16, 18	D8S1179	15 (A->G)-> 16 (A->G)
32	UNTHSC0032-M0309A1	11, 13	28, 30	15 (G->A), 16	11, 12	10, 11	13 (A->G), 14 (A->G)	15, 15 (G->A)		
32	UNTHSC0032-M0309B1	12 (A->T), 14	28, 30	14 (G->A), 19	12, 14	8, 8	11, 13 (A->G)	15, 17		
32	UNTHSC0032-M0309C1	12 (A->T), 13	30, 30	14 (G->A), 15 (G->A)	12, 15	8, 11	11, 13 (A->G)	15 (G->A), 17	D5S818	14->15
33	UNTHSC0032-M0310A1	12, 13	28, 30	15 (2G->2A), 16 (G->A)	11, 11	11, 12	10, 14	15 (G->A), 17		
33	UNTHSC0032-M0310B1	11, 13	30 (A->G), 33.2	14 (G->A), 15 (G->A)	11, 12	9, 9	12, 16 (A->G)	18, 18		
33	UNTHSC0032-M0310C1	12, 13	28, 33.2	15 (2G->2A), 15 (G->A)	11, 12	9, 12	10, 16 (A->G)	17, 17	vWA	18->17

Table 6, continued. Seven-locus profiles for members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Locus	Change
34	UNTHSC0032-M0329B1	11 (A->T), 12	28, 29	16 (2G->2A), 17 (G->A)	8 (G->T), 12 (G->T)	10, 11	13, 16 (A->G)	14 (T->C), 16		
34	UNTHSC0032-M0329C1	11, 13	28, 31.2 (G->A)	15 (G->A), 15 (G->A)	7, 11	8, 10	12 (A->G), 15 (A->G)	15, 18		
34	UNTHSC0036-M0329A1	11, 11 (A->T)	30, 31.2 (G->A)	15 (G->A), 15 (G->A)	7, 13 (G->T)	8, 13	14 (A->G), 15 (A->G)	15, 17		
34	UNTHSC0036-M0329C2	11, 13	28, 31.2 (G->A)	15 (G->A), 15 (G->A)	12, 13 (G->T)	8, 11	12 (A->G), 15 (A->G)	17, 18		
35	UNTHSC0032-M0330A1	10, 12	28, 31.2	15 (G->A), 17	13, 13 (G->T)	8, 10	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 18		
35	UNTHSC0032-M0330B1	12, 12 (A->T)	27, 28	15 (2G->2A), 17 (G->A)	12, 13 (G->T)	10, 10	13 (A->G), 14 (A->G)	16, 18		
35	UNTHSC0033-M0330C1	12, 12 (A->T)	28, 28	15 (2G->2A), 17	12, 13	10, 11	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 16	D7S820	10->11
36	UNTHSC0033-M0331A1	12, 12	28, 28	14 (2G->2A), 16 (2G->2A)	12, 12 (G->T)	8, 8	14 (A->G), 16 (A->G)	18 (2A->2G), 19		
36	UNTHSC0033-M0331C1	12, 12	28, 32 (A->G)	14 (2G->2A), 16	12, 13	8, 8	15 (A->G), 16 (A->G)	18, 19	D5S818	12->13
36	UNTHSC0036-M0331B1	12, 13	29, 32 (A->G)	15 (2G->2A), 16	8 (G->T), 12	8, 8	13 (A->G), 15 (A->G)	18, 19		
37	UNTHSC0033-M0332A1	11, 13	31.2, 33.2	15 (G->A), 16 (G->A)	9 (G->T), 11 (G->T)	10, 10 (T->A)	12 (A->G), 14	15, 16		
37	UNTHSC0033-M0332B1	9, 12 (A->T)	28, 30	15 (G->A), 16 (G->A)	12, 13	8, 10	12 (A->G), 14	16, 16		
37	UNTHSC0033-M0332C1	11, 12 (A->T)	28, 32.2	16 (G->A), 16 (G->A)	11 (G->T), 13	8, 10 (T->A)	12 (A->G), 12 (A->G)	15, 16	D21S11	31.2->32.2 or 33.2->32.2
38	UNTHSC0033-M0333A1	8, 11 (A->T)	30 (A->G), 30 (A->G)	14 (G->A), 16	11, 11	9, 11	10, 12	14 (A->G + 2T->2C), 16		
38	UNTHSC0033-M0333B1	8, 11	31, 31.2	14 (G->A), 16 (G->A)	13 (G->T), 13 (G->T)	11, 11	no data	17, 17		
38	UNTHSC0033-M0333C1	8, 11 (A->T)	30 (A->G), 31	16, 16 (G->A)	12, 13 (G->T)	9, 11	12, 12	14 (A->G + 2T->2C), 15	D5S818	11->12
39	UNTHSC0033-M0335A1	12, 12 (A->T)	28, 31.2	15 (G->A), 18	8 (G->T), 12	8, 11 (T->A)	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 15		
39	UNTHSC0033-M0335B1	11, 12 (A->T)	32 (A->G), 32.2	15 (G->A), 17	11, 13 (G->C)	9, 9	14 (A->G), 14 (A->G)	15, 18 (G->A)		
39	UNTHSC0033-M0335C1	12 (A->T), 12 (A->T)	31.2, 32.2	15 (G->A), 17	8 (G->T), 13 (G->C)	11 (T->A), 11 (T->A)	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17 (G->A)	vWA	18 (G->A)-> 17 (G->A)
40	UNTHSC0033-M0336A1	8, 12 (A->T)	29, 31	14 (G->A), 18	7, 12	11, 13	14 (A->G), 14 (A->G)	16, 17		
40	UNTHSC0033-M0336B1	9, 12 (A->T)	30 (A->G), 32.2	17, 17	11, 12	10, 12	15 (A->G), 15 (A->G)	16, 18		
40	UNTHSC0033-M0336C1	12 (A->T), 12 (A->T)	29, 32.2	14 (G->A), 17	7, 12	10, 11	14 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 17		
41	UNTHSC0033-M0339A1	11 (A->T), 13	30 (A->G), 31.2	16 (G->A), 18	10, 11	11 (T->A), 12	8, 10	16, 17		
41	UNTHSC0033-M0339B1	11, 12 (A->T)	29 (A->G), 30 (A->G)	17 (G->A), 17 (G->A)	11, 13	11, 12 (T->A)	10, 13 (A->G)	14 (A->G + 2T->2C), 20		
41	UNTHSC0033-M0339C1	11 (A->T), 12 (A->T)	29 (A->G), 30 (A->G)	16 (G->A), 17 (G->A)	10, 13	11, 12	8, 13 (A->G)	16, 19	vWA	20->19
42	UNTHSC0033-M0341A1	11 (A->T), 12	29, 31 (G->A)	15 (2G->2A), 17	11, 12	10, 11	13 (A->G), 16 (A->G)	16, 18		
42	UNTHSC0033-M0341B1	11, 11 (A->T)	27, 29	15 (2G->2A), 16 (G->A)	12, 13 (G->C)	11, 11	14 (A->G), 14 (A->G)	15 (G->A), 19 (G->A)		
42	UNTHSC0033-M0341C1	11, 11 (A->T)	27, 29	15 (2G->2A), 17	11, 13 (G->C)	11, 11	13 (A->G), 14 (A->G)	18, 18 (G->A)	vWA	19 (G->A)-> 18 (G->A)
43	UNTHSC0033-M0342A1	8, 12 (A->T)	29, 31.2	13 (2G->2A), 16 (2G->2A)	11, 13	11, 11	14 (A->G), 14 (A->G)	15 (G->A), 19		
43	UNTHSC0033-M0342B1	11, 14	28, 31.2	14 (G->A), 16 (G->A)	13 (G->T), 14	10, 10	14 (A->G), 14 (A->G)	16, 19		
43	UNTHSC0033-M0342C1	8, 14	28, 29	16 (2G->2A), 16 (G->A)	11, 13 (G->T)	10, 11	13 (A->G), 14 (A->G)	15 (G->A), 16	D8S1179	14 (A->G)-> 13 (A->G)
44	UNTHSC0033-M0343A1	8, 12 (A->T)	31, 32.2	15 (G->A), 17 (A->G)	12, 12	10, 10	11, 13	17, 17 (G->A)		
44	UNTHSC0033-M0343B1	11, 14	29, 31	14 (G->A), 16 (G->A)	11, 12	9, 12 (T->A)	14 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17		
44	UNTHSC0033-M0343C1	8, 9	29, 30 (A->G)	17 (A->G), 17 (G->A)	12, 13	10, 11	11, 13	14 (A->G + 2T->2C), 17		
45	UNTHSC0033-M0344A1	9, 9	34.2, 34.2	15 (G->A), 15 (G->A)	11, 11 (G->T)	11, 12	13 (A->G), 14 (A->G)	14 (T->C), 17		
45	UNTHSC0033-M0344B1	9, 11 (A->T)	31, 31.2	15 (G->A), 16 (2G->2A)	11, 12	10, 12	13 (A->G), 14 (A->G)	16, 16 (G->A)		
45	UNTHSC0033-M0344C2	9, 11 (A->T)	31, 31	15 (G->A), 15 (G->A)	11, 11	10, 12	13 (A->G), 13 (A->G)	14 (T->C), 16		
46	UNTHSC0033-M0345A1	12, 12	28, 31	16 (2G->2A), 16 (2G->2A)	13, 13	9, 10	11, 16 (A->G)	14 (A->G + 2T->2C), 15 (G->A)		
46	UNTHSC0033-M0345B1	11 (A->T), 12	27 (A->G), 30	15 (G->A), 16 (G->A)	11, 12 (G->T)	9, 11	14 (A->G), 14 (A->G)	19, 19		
46	UNTHSC0033-M0345C1	12, 12	30, 31	15 (G->A), 16 (2G->2A)	11, 13	9, 11	14 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 20	vWA	19->20
47	UNTHSC0033-M0346A1	13, 14	28, 30 (A->G)	15 (G->A), 17 (G->A)	11, 11	10, 12	14 (A->G), 15 (A->G)	16, 17		
47	UNTHSC0033-M0346B1	10, 13	29, 30 (A->G)	15 (G->A), 17	11, 11	10, 13	13, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
47	UNTHSC0033-M0346C1	12, 13	30 (A->G), 30 (A->G)	17, 17 (G->A)	11, 11	12, 13	13, 14 (A->G)	14 (A->G + 2T->2C), 16	D13S317	13->12
48	UNTHSC0033-M0347A1	8, 12	29, 29	15 (G->A), 16	9 (G->T), 11 (G->T)	8, 9	12, 13 (A->G)	17 (G->A), 18		
48	UNTHSC0033-M0347C1	11 (A->T), 12	28, 29	15 (G->A), 15 (G->A)	11 (G->T), 12	9, 9	12, 16 (A->G)	18, 18 (G->A)	vWA	19 (G->A)-> 18 (G->A)
48	UNTHSC0036-M0347B1	11 (A->T), 12	28, 30 (A->G)	15 (G->A), 16 (G->A)	11, 12	8, 9	15 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 19 (G->A)		
49	UNTHSC0033-M0348B1	12, 13 (A->T)	30 (A->G), 31	15, 15 (G->A)	13, 14 (G->T)	10, 10	14 (A->G), 14 (A->G)	15, 17 (G->A)		
49	UNTHSC0033-M0348C1	12, 12	30 (A->G), 31	15, 15 (G->A)	11, 13	8, 10	12, 14 (A->G)	17 (G->A), 17 (G->A)		
49	UNTHSC0036-M0348A1	12, 14	30 (A->G), 30 (A->G)	12 (2G->2A), 15 (G->A)	11, 11	8, 11	12, 13 (A->G)	16, 16		
50	UNTHSC0033-M0349A1	11, 14	30.2, 32.2	14 (2G->2A), 17	11, 12 (G->T)	7, 8	12 (A->G), 16 (2A->2G)	16, 17		
50	UNTHSC0033-M0349B1	10 (A->T), 12	29, 31	15, 15 (2G->2A)	11, 13 (G->C)	9, 11	14 (A->G), 16 (A->G)	18, 20		
50	UNTHSC0033-M0349C1	14, 14	29, 30.2	14 (2G->2A), 15 (2G->2A)	12 (G->T), 13 (G->C)	7, 9	14 (A->G), 17 (2A->2G)	16, 18	D8S1179	16 (2A->2G) -> 17 (2A->2G)
51	UNTHSC0033-M0350A1	8, 12	28 (A->G), 29	16 (G->A), 18	11, 12	11 (T->A), 12	12, 13 (A->G)	15 (G->A), 19		
51	UNTHSC0033-M0350B1	11, 11	28, 28	14 (G->A), 15 (2G->2A)	12 (G->T), 12 (G->T)	10, 11	12 (A->G), 14 (A->G)	16, 20		
51	UNTHSC0033-M0350C1	8, 11	28, 28 (A->G)	15 (2G->2A), 16 (G->A)	11, 12 (G->T)	11, 11 (T->A)	12, 12 (A->G)	19, 21	vWA	20->21
52	UNTHSC0033-M0351A1	11 (A->T), 11 (A->T)	30, 32.2	15 (G->A), 17	11, 12	9 (T->C), 13	11, 13	14 (A->G + 2T->2C), 17		
52	UNTHSC0033-M0351B1	11 (A->T), 11 (A->T)	28, 30 (A->G)	16 (G->A), 18	11, 12	9, 10 (T->A)	12, 15 (A->G)	18, 18		
52	UNTHSC0033-M0351C1	11 (A->T), 11 (A->T)	28, 30	16 (G->A), 17	11, 12	9, 13	11, 15 (A->G)	14 (A->G + 2T->2C), 17	vWA	18->17
53	UNTHSC0033-M0352A1	11, 14	30 (A->G), 31	15 (G->A), 16 (2G->2A)	11 (G->T), 12	10, 11	14 (A->G), 15 (A->G)	17, 17 (G->A)		
53	UNTHSC0033-M0352B1	11 (A->T), 12	28, 30 (A->G)	17, 18 (G->A)	12 (G->T), 14	8, 10	10, 14 (A->G)	15, 16		
53	UNTHSC0033-M0352C1	11, 11 (A->T)	30 (A->G), 31	16 (2G->2A), 18 (G->A)	12, 13	10, 11	10, 14 (A->G)	16, 17	D5S818	14->13
54	UNTHSC0033-M0353A1	10 (A->T), 12	28, 29	14 (G->A), 15 (G->A)	12, 12	9, 12	13 (A->G), 14 (A->G)	16, 17		
54	UNTHSC0033-M0353B1	9, 12 (A->T)	29 (G->A), 30 (A->G)	15 (G->A), 18	7, 13	8, 10	13 (A->G), 14 (A->G)	11, 19		
54	UNTHSC0033-M0353C1	10 (A->T), 12 (A->T)	28, 29 (G->A)	14 (G->A), 15 (G->A)	12, 13	8, 12	13 (A->G), 14 (A->G)	16, 20	vWA	19->20
55	UNTHSC0033-M0355A1	12, 12	30 (A->G), 34 (A->G)	15 (2G->2A), 17	11, 13	8, 8	13 (A->G), 15 (A->G)	17, 17		
55	UNTHSC0033-M0355B1	10 (A->T + G->A), 13	28, 32.2	15 (2G->2A), 16 (G->A)	12, 13 (G->C)	10, 11	13, 15 (A->G)	16, 19 (G->A)		
55	UNTHSC0036-M0355C1	12, 13	28, 30 (A->G)	16 (G->A), 17	11, 12	8, 10	12, 15 (A->G)	16, 17	D8S1179	13->12
56	UNTHSC0033-M0356A1	12, 13	29, 30	15 (G->A), 16 (G->A)	12, 12	10, 11	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
56	UNTHSC0033-M0356B1	12, 13	29 (G->A), 30 (A->G)	14 (G->A), 15 (G->A)	12, 12	11 (T->A), 13	13 (A->G), 15	14 (T->C), 16		
56	UNTHSC0033-M0356C1	12, 12	29, 29 (G->A)	14 (G->A), 16 (G->A)	12, 12	11, 13	13 (A->G), 14	14 (A->G + 2T->2C), 16	D8S1179	15->14
57	UNTHSC0033-M0357A1	9, 13	29, 30 (A->G)	15 (G->A), 16 (G->A)	11, 11	9, 11	14 (A->G), 14 (A->G)	16, 20		
57	UNTHSC0033-M0357B1	10 (A->T), 13	29, 30 (A->G)	15 (G->A), 16	11, 12	10, 10	13, 16 (A->G)	16, 17		
57	UNTHSC0033-M0357C1	13, 13	29, 30 (A->G)	15 (G->A), 16 (G->A)	11, 12	9, 10	14 (A->G), 15 (A->G)	16, 20	D8S1179	16 (A->G)-> 15 (A->G)
58	UNTHSC0033-M0358B1	11, 12	29, 33.2	15 (G->A), 16 (G->A)	11, 11	9, 10	14 (A->G), 14 (A->G)	16, 18		
58	UNTHSC0033-M0358C1	9, 11	30 (A->G), 33.2	15 (G->A), 15 (G->A)	7, 11	10, 10	13, 14 (A->G)	16, 17		
58	UNTHSC0036-M0358A1	9, 12	29, 33.2	14 (2G->2A), 15 (G->A)	11, 12	9, 11	13 (A->G), 14 (A->G)	18, 18		
59	UNTHSC0033-M0359A1	8, 12	30 (A->G), 30 (A->G)	14 (G->A), 16	10, 12 (G->T)	9, 12	13, 17 (A->G)	16, 18		
59	UNTHSC0033-M0359C1	8, 12 (A->T)	29 (G->A), 30 (A->G)	14 (G->A), 16 (2G->2A)	10, 11	9, 12	13, 14 (A->G)	17, 18		
59	UNTHSC0033-M0359C2	12, 12 (A->T)	30 (A->G), 30 (A->G)	16, 16 (2G->2A)	12 (G->T), 13	9, 9	14 (A->G), 17 (A->G)	16, 17	D5S818	12->13
59	UNTHSC0036-M0359B1	11 (A->T), 12 (A->T)	29 (G->A), 30 (A->G)	14 (G->A), 16 (2G->2A)	11, 12	9, 12	14 (A->G), 14 (A->G)	15 (G->A), 17		
60	UNTHSC0034-M0361A1	9, 11 (A->T)	30 (A->G), 32.2	14 (G->A), 16 (G->A)	7, 12	10, 11	14, 15 (A->G)	13 (G->A + C->T), 17		
60	UNTHSC0034-M0361B1	9, 11	30, 30 (A->G)	16 (G->A), 17 (G->A)	12 (G->T), 13	11, 11	12, 15 (A->G)	15 (G->A), 17		
60	UNTHSC0036-M0361C1	11, 11 (A->T)	30 (A->G), 32.2	16 (G->A), 16 (G->A)	12, 14	10, 11	12, 14	15 (G->A), 17	D5S818	13->14
61	UNTHSC0034-M0363A1	13, 14	29, 33.2	15 (G->A), 15 (G->A)	10, 10 (G->T)	10, 11	14, 14 (A->G)	16, 18		
61	UNTHSC0034-M0363B1	12, 14	31.2, 32.2	15 (G->A), 15 (G->A)	12, 13	10, 10	14 (A->G), 15 (A->G)	16, 17		
61	UNTHSC0034-M0363C1	9, 13	29, 29	15 (G->A), 16 (G->A)	10, 11	10, 11	10, 14	16, 18		
61	UNTHSC0034-M0363C2	12, 14	31.2, 33.2	15 (G->A), 15 (G->A)	10 (G->T), 12	10, 11	13, 14 (A->G)	17, 18	D8S1179	14->13
61	UNTHSC0034-M0363C3	12, 13	29, 31.2	15 (G->A), 15 (G->A)	10, 13	10, 10	14 (A->G), 14 (A->G)	17, 18		
62	UNTHSC0034-M0364B1	11, 14	29, 30 (A->G)	16 (G->A), 17	11, 12	8, 11	13 (A->G), 15 (A->G)	15 (G->A), 16		
62	UNTHSC0034-M0364C1	9, 13	28, 30 (A->G)	15 (G->A), 17	11, 11	8, 8	13, 13 (A->G)	16, 17	D13S317	14->13
62	UNTHSC0036-M0364A1	9, 9	28, 30	15 (2G->2A), 15 (G->A)	11, 11	8, 9	10, 13	16, 17		
63	UNTHSC0034-M0365A1	13, 14	29, 31.2	16 (G->A), 16 (G->A)	11 (G->T), 12 (G->T)	7, 11	10, 16 (A->G)	17, 19 (A->G)		
63	UNTHSC0034-M0365B1	11 (A->T), 14	28, 32.2	15 (G->A), 17 (G->A)	9 (G->T), 12	8, 11	13, 14 (A->G)	16, 16		
63	UNTHSC0034-M0365C1	14, 14	29, 32.2	16 (G->A), 17 (G->A)	9 (G->T), 12 (G->T)	8, 11	12, 16 (A->G)	16, 19 (A->G)	D8S1179	13->12
63	UNTHSC0034-M0365C2	11 (A->T), 14	29, 32.2	16 (G->A), 17 (G->A)	11 (G->T), 12	8, 11	10, 13	16, 17		
64	UNTHSC0034-M0368A1	13, 14	31.2, 33.2	14 (G->A), 15 (G->A)	7, 11	9, 11	11, 14 (A->G)	14 (A->G + 2T->2C), 17		
64	UNTHSC0034-M0368B1	12, 12 (A->T)	30 (A->G), 32 (A->G)	17, 18	12 (G->T), 13	11, 11	13, 13 (A->G)	16, 19		
64	UNTHSC0034-M0368C1	12, 13	30 (A->G), 31.2	15 (G->A), 17	7, 14	9, 11	11, 13	14 (A->G + 2T->2C), 16	D5S818	13->14
65	UNTHSC0034-M0370A1	12 (A->T), 13	no data	16 (G->A), 17 (G->A)	11, 12	11, 14	11, 13 (A->G)	17, 17		
65	UNTHSC0034-M0370B1	11 (A->T), 14	28 (G->A), 30	15 (G->A), 18	12 (G->T), 13	10, 10	12, 14 (A->G)	15 (G->A), 18		
65	UNTHSC0034-M0370C1	13, 14	29, 30	16 (G->A), 18	11, 12 (G->T)	10, 14	11, 14 (A->G)	17, 17	vWA	18->17
66	UNTHSC0034-M0371A1	9, 11 (A->T)	28, 30 (A->G)	15 (G->A), 18	11, 11	9, 13 (T->A)	15 (A->G), 15 (A->G)	17, 17		
66	UNTHSC0034-M0371B1	8, 9	28, 29	15 (2G->2A), 16	11, 12 (G->T)	9, 13 (T->A)	10, 13 (A->G)	17, 17		
66	UNTHSC0034-M0371C1	8, 11 (A->T)	28, 30 (A->G)	15 (2G->2A), 18	11, 11	9, 13 (T->A)	13 (A->G), 15 (A->G)	16, 17	vWA	17->16

Table 6, continued. Seven-locus profiles for members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Mutated locus	From -> To
67	UNTHSC0034-M0372A1	12, 12 (A->T)	29, 30 (A->G)	14 (G->A), 17	8 (G->T), 13	11, 13	15 (A->G), 15 (A->G)	15, 15		
67	UNTHSC0034-M0372B1	11 (A->T), 12	27 (A->G), 30 (A->G)	14 (G->A), 19	11, 14	10, 13	14 (A->G), 14 (A->G)	17, 19		
67	UNTHSC0034-M0372C1	11 (A->T), 12	30 (A->G), 30 (A->G)	14 (G->A), 17	11, 13	10, 13	14 (A->G), 15 (A->G)	16, 19	vWA	15->16
68	UNTHSC0034-M0373A1	10, 12	29 (G->A), 30.2	15 (G->A), 16 (G->A)	11, 12	10, 10	14 (A->G), 14 (A->G)	15, 16		
68	UNTHSC0034-M0373B1	11 (A->T), 13	30 (A->G), 31.2	15 (G->A), 17	11, 11	11, 11 (T->A)	13, 13 (A->G)	14 (A->G + 2T->2C), 17		
68	UNTHSC0034-M0373C1	11 (A->T), 12	29 (G->A), 31.2	16 (G->A), 17	11, 11	10, 11	13, 14 (A->G)	16, 16	vWA	17->16
69	UNTHSC0034-M0374A1	12 (A->T), 12 (A->T)	28, 32.2	14 (G->A), 15 (G->A)	11, 12 (G->T)	8, 11	13, 13 (A->G)	16, 18		
69	UNTHSC0034-M0374B1	12, 12 (A->T)	30, 34.2	15 (G->A), 16 (G->A)	8 (G->T), 11	9 (T->A), 11	12, 13 (A->G)	16, 16		
69	UNTHSC0034-M0374C1	12 (A->T), 13 (A->T)	30, 32.2	15 (G->A), 16 (G->A)	11, 12 (G->T)	9 (T->A), 11	12, 13	16, 16	D13S317	12 (A->T)-> 13 (A->T)
70	UNTHSC0034-M0375A1	12 (A->T), 13	30 (A->G), 32.2	15, 18	12, 12	9, 10	12, 13	16 (G->A), 21 (2A->2G)		
70	UNTHSC0034-M0375B1	11, 12	29, 30 (A->G)	16, 16	11, 11	11, 12	12, 14 (A->G)	17, 17		
70	UNTHSC0034-M0375C1	11, 12	28, 28	15 (G->A), 16	11, 12 (G->T)	8, 11	12 (A->G), 16 (A->G)	16, 19 (A->G)		
71	UNTHSC0034-M0376A1	12 (A->T), 14	29, 29 (G->A)	16, 18	12, 13	11, 13	14 (A->G), 16 (2A->2G)	14 (2A->2G + 2T->2C), 14 (2A->2G + 2T->2C)		
71	UNTHSC0034-M0376B1	10, 13	28, 29 (G->A)	16 (G->A), 17 (G->A)	12, 12 (G->T)	11, 11	12, 14	15, 15		
71	UNTHSC0034-M0376C2	12 (A->T), 13	29 (G->A), 29 (G->A)	16, 17 (G->A)	12, 12 (G->T)	11, 11	14 (A->G), 15	13 (C->T), 15	D8S1179	14->15
71	UNTHSC0036-M0376C1	13, 14	28, 29 (G->A)	16, 16 (G->A)	12 (G->T), 13	11, 13	12, 14 (A->G)	14 (2A->2G + 2T->2C), 15		
72	UNTHSC0034-M0377B1	11 (A->T), 11 (A->T)	31, 31.2	16 (2G->2A), 17	12 (G->T), 13	8, 11	14 (A->G), 14 (A->G)	14 (T->C), 15		
72	UNTHSC0034-M0377C1	11 (A->T), 13	27, 31	14 (2G->2A), 16 (2G->2A)	13, 13 (G->T)	8, 11	12 (A->G), 15 (A->G)	13 (C->T), 15		
72	UNTHSC0036-M0377A1	11, 13	27, 28	14 (2G->2A), 15 (2G->2A)	11, 13 (G->T)	10, 11	15 (2A->2G), 15 (A->G)	13 (C->T), 14 (A->G + 2T->2C)		
73	UNTHSC0034-M0378A1	11 (A->T), 12	29 (A->G), 30.2	16, 16 (G->A)	13, 13 (G->T)	9, 10	10, 13 (A->G)	14 (A->G + 2T->2C), 16 (G->A)		
73	UNTHSC0034-M0378B1	12 (A->T), 13	30 (A->G), 31	15, 18	12, 12	9, 10	12, 12	16 (G->A), 21 (2A->2G)		
73	UNTHSC0034-M0378C1	11 (A->T), 13	30.2, 31	15, 16	12, 13 (G->T)	10, 10	12, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	vWA	21 (2A-.2G)-> 20 (2A->2G)
74	UNTHSC0034-M0379A1	12, 13 (A->T)	31.2, 32.2	15 (2G->2A), 15 (G->A)	12, 12 (G->T)	11, 12	13, 13 (A->G)	16 (G->A), 17		
74	UNTHSC0034-M0379B1	8, 13	29 (G->A), 30	17 (G->A), 18	11, 13	10, 11	11, 12	15 (G->A), 19		
74	UNTHSC0034-M0379C1	12, 12	no data	15 (G->A), 18	12 (G->T), 13	11, 11	11, 13	15 (G->A), 16 (G->A)	D13S317	13->12
75	UNTHSC0034-M0380A1	11 (A->T), 13	32.2, 32.2	15, 18	9 (G->T), 12 (G->T)	10, 11	10, 12 (A->G)	18, 18		
75	UNTHSC0034-M0380B1	12, 13	28, 35 (T->G)	16 (G->A), 16 (G->A)	12, 13	10, 12 (T->A)	12 (A->G), 15 (A->G)	16, 18		
75	UNTHSC0034-M0380C1	12, 12	28, 31	15, 16 (G->A)	9 (G->T), 13	10, 12 (T->A)	10, 15 (A->G)	18, 18	D13S317	13->12
76	UNTHSC0034-M0381A1	12, 13	28, 28	16, 16 (2G->2A)	10, 12	6, 10	13 (A->G), 14 (A->G)	15, 16		
76	UNTHSC0034-M0381B1	11, 12 (A->T)	28, 30 (A->G)	15 (G->A), 18	10, 12	11, 13	12, 13 (A->G)	17, 17		
76	UNTHSC0034-M0381C1	13, 13 (A->T)	28, 28	16, 18	12, 12	10, 13	13 (A->G), 14 (A->G)	16, 17	D13S317	12 (A->T)-> 13 (A->T)
77	UNTHSC0034-M0382A1	11 (A->T), 12	28, 30 (A->G)	15 (G->A), 17 (2G->2A)	10, 11	8, 10	15 (2A->2G), 15 (2A->2G)	15, 18		
77	UNTHSC0034-M0382B1	11, 14	28, 35	16 (2G->2A), 16 (2G->2A)	12, 13	10, 11	14 (A->G), 14 (A->G)	17, 19		
77	UNTHSC0034-M0382C1	12, 14	28, 35	16 (2G->2A), 17 (2G->2A)	10, 13	10, 11	14 (A->G), 15 (2A->2G)	15, 18	vWA	17->18 or 19->18
78	UNTHSC0034-M0385A1	11 (A->T), 12	30 (A->G), 31.2	15 (2G->2A), 15 (2G->2A)	11, 12 (G->T)	8, 9	13 (A->G), 13 (A->G)	17, 17 (G->A)		
78	UNTHSC0034-M0385B2	11, 12 (A->T)	31.2, 31.2	15 (G->A), 17 (G->A)	12, 12 (G->T)	8, 11	11, 13 (A->G + C->G)	17, 18		
78	UNTHSC0034-M0385C1	11, 11 (A->T)	30 (A->G), 31.2	15 (2G->2A), 15 (G->A)	12 (G->T), 12 (G->T)	9, 11	12, 13 (A->G)	17, 18	D8S1179	11->12
79	UNTHSC0034-M0386A1	11, 11	30 (A->G), 31.2	9 (G->A), 15 (G->A)	13, 13	9, 9	11, 14 (A->G)	18, 18 (G->A)		
79	UNTHSC0034-M0386B1	9, 11 (A->T)	28, 30 (A->G)	15 (G->A), 17 (G->A)	10, 13	7, 10	14 (A->G), 16 (A->G)	16, 18 (A->G)		
79	UNTHSC0034-M0386C1	11, 11 (A->T)	30 (A->G), 31.2	15 (G->A), 17 (G->A)	13, 14	9, 10	14 (A->G), 16 (A->G)	18 (A->G), 18 (G->A)	D5S818	13->14
80	UNTHSC0034-M0388A1	11, 11 (A->T)	29, 33.2	15 (G->A), 16	12, 12 (G->T)	11, 12	10, 14 (A->G)	17, 18		
80	UNTHSC0034-M0388B1	11 (A->T), 12	28, 29	14 (G->A), 17 (G->A)	11, 11	8, 11	10, 12	14 (A->G + 2T->2C), 17		
80	UNTHSC0036-M0388C1	11, 11 (A->T)	28, 28	14 (G->A), 16	11, 12	11, 12	10, 14 (A->G)	14 (A->G + 2T->2C), 18	D21S11	29->28
81	UNTHSC0034-M0389A1	11, 11 (A->T)	29, 32.2	15 (G->A), 16	11, 12	10, 12	13 (A->G), 14	17, 17		
81	UNTHSC0034-M0389B1	11 (A->T), 13	29, 30.2	14 (2G->2A), 15 (2G->2A)	11, 11	10, 10	13 (A->G), 14 (A->G)	16, 18 (G->A)		
81	UNTHSC0034-M0389C2	11, 11 (A->T)	29, 30.2	15 (2G->2A), 16	11, 12	10, 12	13 (A->G), 14 (A->G)	17, 19 (G->A)	vWA	18 (G->A)-> 19 (G->A)
81	UNTHSC0036-M0389C1	11, 11 (A->T)	27, 32.2	16, 16 (G->A)	11, 12	10, 13	14, 16 (A->G)	17, 19 (A->G)		
82	UNTHSC0035-M0409A1	9, 10 (A->T)	29 (G->A), 31.2	15 (G->A), 15 (G->A)	11, 11 (G->T)	8, 11 (T->A)	14, 14 (A->G)	15 (A->G + 2T->2C), 18		
82	UNTHSC0035-M0409B1	9, 12	30, 32.2	17 (G->A), 18	11, 13	8, 12 (T->A)	13 (A->G), 14	16, 17		
82	UNTHSC0035-M0409C1	9, 10 (A->T)	29 (G->A), 32.2	15 (G->A), 17 (G->A)	11, 13	8, 11 (T->A)	13 (A->G), 14	15 (A->G + 2T->2C), 17		
82	UNTHSC0035-M0409C2	10 (A->T), 12	31.2, 32.2	15 (G->A), 18	11, 13	8, 8	13 (A->G), 14 (A->G)	18, 18	vWA	17->18
82	UNTHSC0035-M0409C3	9, 10 (A->T)	31.2, 32.2	15 (G->A), 17 (G->A)	11, 11	8, 11 (T->A)	14, 14 (A->G)	16, 18		
82	UNTHSC0035-M0409C4	10 (A->T), 12	30, 31.2	15 (G->A), 18	11, 11	8, 11 (T->A)	14, 14 (A->G)	15 (A->G + 2T->2C), 16		
83	UNTHSC0035-M0425A1	8, 12 (A->T)	30, 31	15 (G->A), 15 (G->A)	9 (G->T), 12 (G->T)	11, 11	13 (A->G), 14 (A->G)	16, 17		
83	UNTHSC0035-M0425B1	11 (A->T), 12	30 (A->G), 31.2 (G->A)	16, 17 (G->A)	7, 11	11, 12	12, 14 (A->G)	17, 18		
83	UNTHSC0035-M0425C1	12 (A->T), 12 (A->T)	30, 30 (A->G)	15 (G->A), 15 (G->A)	11, 12 (G->T)	11, 11	13 (A->G), 14 (A->G)	16, 16		
84	UNTHSC0035-M0392A1	12, 14	28, 28	15 (2G->2A), 16	11, 12	10, 12	14 (A->G), 15 (A->G)	16 (T->C), 18		
84	UNTHSC0035-M0392B1	11 (A->T), 12 (A->T)	28, 28	15 (G->A), 16 (G->A)	12 (G->T), 12 (G->T)	10, 10	14 (A->G), 15 (A->G)	15, 17		
84	UNTHSC0035-M0392C1	11 (A->T), 12	28, 33.2	15 (2G->2A), 16 (G->A)	10, 12	9, 12	15 (A->G), 15 (A->G)	16 (T->C), 17		
85	UNTHSC0035-M0394A1	11, 14	29 (G->A), 31	15 (G->A), 18	7, 11	10, 10	10, 15 (A->G)	15 (A->G + 2T->2C), 18		
85	UNTHSC0035-M0394B1	12, 12 (A->T)	31, 32.2	16 (2G->2A), 18 (G->A)	12, 13 (G->C)	10, 11	11 (A->G), 14 (A->G)	16, 17		
85	UNTHSC0035-M0394C1	13 (A->T), 14	29 (G->A), 31	16 (2G->2A), 18	7, 12	10, 10	11 (A->G), 15 (A->G)	16, 18	D13S317	12 (A->T)-> 13 (A->T)
86	UNTHSC0035-M0395A1	8, 11	32.2 (A->G), 33.2	15 (G->A), 17 (G->A)	11, 12	11, 12 (T->A)	12, 15 (A->G)	16, 17		
86	UNTHSC0035-M0395B1	8, 11	31, 32.2 (A->G)	15 (G->A), 17 (G->A)	11, 12	11, 12 (T->A)	12, 12 (A->G)	17, 18		
86	UNTHSC0035-M0395C1	11, 11	32.2 (A->G), 33.2	15 (G->A), 17 (G->A)	11, 11	11, 12 (T->A)	13, 15 (A->G)	17, 18	D8S1179	12->13
87	UNTHSC0035-M0396A1	9, 10	30 (A->G), 30 (A->G)	14 (G->A), 16 (G->A)	11, 11	11, 12	13, 13 (A->G)	16, 18		
87	UNTHSC0035-M0396B1	9, 9	30, 32.2	15 (G->A), 18	11, 12	11, 11 (T->A)	13, 13 (A->G)	15 (G->A), 17		
87	UNTHSC0035-M0396C2	9, 9	30 (A->G), 32.2	16 (G->A), 18	11, 11	11, 11 (T->A)	13, 13 (A->G)	16 (G->A), 18	vWA	15 (G->A)-> 16 (G->A)
88	UNTHSC0035-M0397A1	9, 10 (A->T)	28, 30 (A->G)	15 (G->A), 16 (G->A)	9 (G->T), 11	8, 12	10, 13 (A->G)	16, 18		
88	UNTHSC0035-M0397B1	11 (A->T), 13	29, 31.2	15 (G->A), 16 (G->A)	10, 11	11, 11 (T->A)	13, 15 (A->G)	17, 17		
88	UNTHSC0035-M0397C1	10 (A->T), 13	27 (A->G), 31.2	15 (G->A), 15 (G->A)	9 (G->T), 10	11, 12	10, 15 (A->G)	16, 17	D21S11	28->27
89	UNTHSC0035-M0398A1	11 (A->T), 12	31 (G->A), 32.2	14 (2G->2A), 17	13 (G->C), 13 (G->C)	9, 12	12 (A->G), 13 (A->G)	15, 15		
89	UNTHSC0035-M0398B1	11, 12	28, 31.2	14 (G->A), 15 (G->A)	12, 12 (G->T)	9, 11	14 (A->G), 14 (A->G)	15, 17		
89	UNTHSC0035-M0398C1	11 (A->T), 13	31 (G->A), 31.2	15 (G->A), 17	12, 13 (G->T)	9, 12	13 (A->G), 14 (A->G)	15, 15	D13S317	12->13
90	UNTHSC0035-M0400A1	8, 11 (A->T)	30, 31	15 (G->A), 18	11, 12	8, 10	13, 15 (A->G)	14 (A->G + 2T->2C), 17		
90	UNTHSC0035-M0400B1	12, 12 (A->T)	30 (A->G), 32	15 (2G->2A), 18	8 (G->T), 10	11, 13	16 (A->G), 16 (A->G)	18, 20 (A->G)		
90	UNTHSC0035-M0400C1	8, 12	31, 32	15 (G->A), 18	10, 12	8, 11	15 (A->G), 17 (A->G)	17, 18	D8S1179	16 (A->G)-> 17 (A->G)
91	UNTHSC0035-M0401A1	12, 12 (A->T)	27, 30 (A->G)	15 (2G->2A), 16	10, 11 (G->T)	8, 10	11, 13 (A->G)	19, 19		
91	UNTHSC0035-M0401B1	13, 13	31.2, 32.2	14 (2G->2A), 15 (G->A)	12, 12	11, 13 (T->A)	15 (2A->2G + A->G), 16 (A->G)	15, 16 (G->A)		
91	UNTHSC0035-M0401C1	12, 12 (A->T)	27, 32.2	14 (2G->2A), 15 (2G->2A)	11 (G->T), 12	10, 13 (T->A)	11, 15 (2A->2G + A->G)	15, 19	D13S317	13->12
92	UNTHSC0035-M0402A1	10 (A->T), 12	29, 32.2	15 (G->A), 16	11, 12	12, 13	15 (A->G), 16 (A->G)	16, 16 (G->A)		
92	UNTHSC0035-M0402B1	8, 10 (A->T)	31.2, 32 (A->G)	15 (G->A), 17	11, 11	9, 12 (T->A)	11, 13 (A->G)	16, 17		
92	UNTHSC0035-M0402C1	10 (A->T), 12	31.2, 32.2	15 (G->A), 17	11, 11	9, 13	13 (A->G), 15 (A->G)	16, 16		
93	UNTHSC0035-M0403A1	12, 13 (A->T)	29, 33.2	15 (G->A), 16 (G->A)	11, 13	10, 11	12, 15 (2A->2G)	17, 18		
93	UNTHSC0035-M0403B1	12, 12	30 (A->G), 35.2	16, 16 (G->A)	7, 11	10, 11	10, 15 (A->G)	17, 19		
93	UNTHSC0035-M0403C1	11, 13 (A->T)	30 (A->G), 33.2	15 (G->A), 16 (G->A)	11, 13	10, 10	10, 12	17, 17	D13S317	12->11
94	UNTHSC0035-M0404A1	12, 12 (A->T)	32, 32.2	15 (G->A), 16 (2G->2A)	11, 12	10, 12	13 (A->G), 13 (A->G)	17 (G->A), 19		
94	UNTHSC0035-M0404B1	8, 12	29 (A->G), 31	15 (2G->2A), 18	9 (G->T), 11	9, 9	10, 13 (A->G)	16, 18		
94	UNTHSC0035-M0404C1	12, 12 (A->T)	31, 32	16 (2G->2A), 18	9 (G->T), 12	9, 12	11, 13 (A->G)	16, 17 (G->A)	D8S1179	10->11
95	UNTHSC0035-M0405A1	10 (A->T), 13	29, 31.2	16 (G->A), 18 (G->C)	11, 11	12, 14 (T->A)	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 19		
95	UNTHSC0035-M0405B1	9, 11 (A->T)	30, 32.2	16 (G->A), 17	9 (G->T), 11	10 (T->A), 12	13 (A->G), 14	15 (G->A), 17		
95	UNTHSC0035-M0405C1	9, 13	29, 30	16 (G->A), 18 (G->C)	11, 11	12, 14 (T->A)	13 (A->G), 14	14 (A->G + 2T->2C), 16 (G->A)	vWA	15 (G->A)-> 16 (G->A)
95	UNTHSC0035-M0405C2	8, 13	29, 31.2	15 (G->A), 16 (G->A)	11, 12	11, 14 (T->A)	12, 13 (A->G)	16, 19		
95	UNTHSC0035-M0405C3	9, 10 (A->T)	29, 30	17, 18 (G->C)	11, 11	10 (T->A), 12	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 15 (G->A)		
96	UNTHSC0035-M0406A1	11, 11 (A->T)	30 (A->G), 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	9, 11	13 (A->G), 14 (A->G)	16, 18		
96	UNTHSC0035-M0406B1	9, 9	28, 29	15 (G->A), 18	11, 11	8, 12 (T->A)	11, 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C1	9, 11	28, 30 (A->G)	15 (G->A), 18	11, 11	9, 12 (T->A)	11, 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C2	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	11, 9	11, 13 (A->G)	16 (G->A), 18		
96	UNTHSC0035-M0406C3	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	7, 11	11, 12 (T->A)	13 (A->G), 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C4	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	8, 9	14 (A->G), 14 (A->G)	16, 17 (G->A)	D8S1179	16 (A->G)-> 17 (A->G)
97	UNTHSC0036-82001A1	11 (A->T), 12	28, 30	14 (G->A), 17 (2G->2A)	11, 12 (G->T)	8, 12	12, 12 (A->G)	16, 18		
97	UNTHSC0036-82001B1	8, 8	28, 34 (2A->2G)	15 (G->A), 16 (G->A)	11, 12	8, 10	11, 13	15, 17 (G->A)		
97	UNTHSC0036-82001C1	8, 12	28, 34 (2A->2G)	14 (G->A), 15 (G->A)	11, 11	8, 10	11, 12	15, 18		
97	UNTHSC0036-82001C2	8, 12	28, 30	14 (G->A), 16 (G->A)	11, 12 (G->T)	8, 10	11, 12 (A->G)	15, 16		
97	UNTHSC0036-82001C3	8, 12	28, 30	14 (G->A), 16 (G->A)	12, 12 (G->T)	8, 12	12, 13	15, 18		
97	UNTHSC0036-82001C4	8, 12	28, 34 (2A->2G)	16 (G->A), 17 (2G->2A)	11, 11	8, 8	12 (A->G), 13	15, 18		

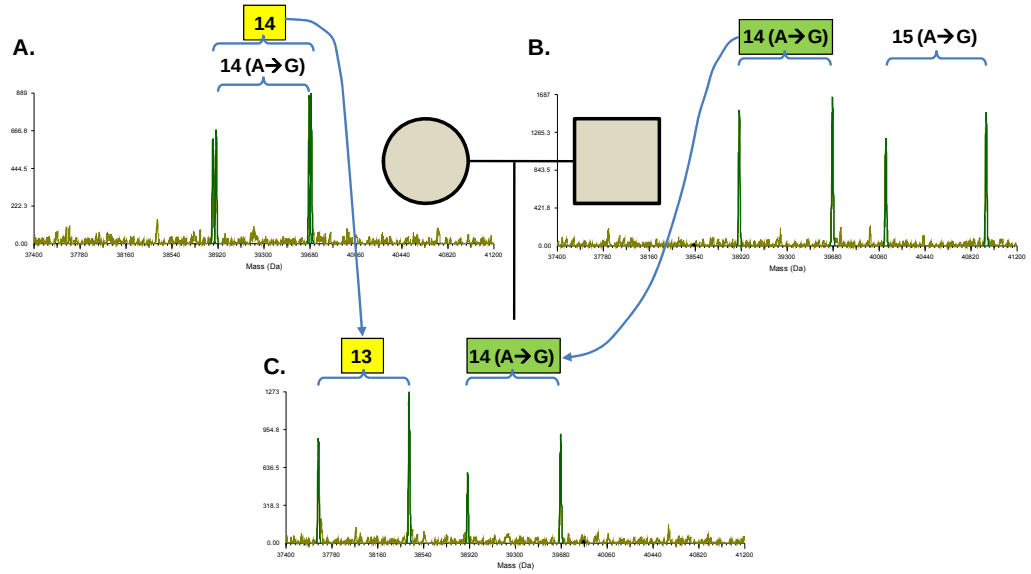


Figure 10. D8S1179 genotypes for a mother-father-child trio. The mother (A.) has genotype 14, 14 (A→G). The father (B) has genotype 14 (A→G), 15 (A→G). The child (C) has genotype 13, 14 (A→G). The simplest explanation for this observation is that the father contributed the non-mutated 14 (A→G) allele and the mother in fact contributed an allele 14 that mutated to a 13 in the child.

Specific Aim 5: Continued development of transferable analysis software with an intuitive user interface

Over the course of this project, the processing of raw mass spectra to produce deconvolved spectral traces and numerical mass and intensity values that are utilized during the data analysis of STR and Y-STR assay outputs has progressed from a manually-triggered interface running within Matlab to a fully-automated, completely native code-based processing application written in C# (no Matlab interface or runtime environment required) that requires no user input and is seamlessly integrated in the process of running on a plate on the Ibis instrument. Recently, the processing application has been migrated to a Windows service module that runs as a background process on the data processing server and is invisible to the user. After thermocycling an assay plate that has been registered into the IbisTrack database, the assay plate is placed upon the Ibis T5000 or PLEX-ID instrument and the instrument is started. At the point that all data has been collected for the plate, the automation controller automatically converts the data into a familiar folder-based data output, copies all raw spectral data to a configured output directory, triggers the processing of raw mass spectra into deconvolved mass spectra, generates and output list of masses and signal intensities, and imports the output back into the database linked to the barcode of the assay plate to await analysis and visual QC. This process operates identically for all forensics applications.

The forensics data analysis interface and database has been generalized to allow for the analysis of any base composition or allele-based forensic assay running on the Ibis platform including mtDNA profiling, STRs, Y-STRs or autosomal SNP markers. The processing and analysis mode is easily extendable to other forensic-type analysis such

as microbial SNP or VNTR analysis, as the analysis mode and profiling methodology is essentially the same as that done with human DNA. Functionality to store and retrieve STR and Y-STR profiles directly from the analysis interface has been implemented, as well as an interface to search STR or Y-STR profiles using a stored profile as a query. Abbott Molecular is currently undergoing commercialization of the forensics PLEX-ID system and the forensics analysis software is on the verge of commercialization.

Introduction

Statement of the problem

Short Tandem Repeat (STR) markers have become the human forensic "gold standard" in recent years as the combined information derived from 13 distinct alleles (CODIS 13) provide enough information to statistically unique an individual's DNA signature to 1 in 10^9. While offering extremely high differentiation, the approach is not without limitations. At low copy number it is not uncommon to observe allele "drop out" in which a heterozygous individual is typed as a homozygote because one of the alleles is not detected. Additionally, for highly degraded DNA samples, entire markers may drop out leaving only a few STRs from which to derive a DNA profile. While in some cases a partial profile can be used to include or exclude a potential suspect, there is a need within the forensics community to derive maximal information from degraded DNA samples which yield an incomplete set of STR markers.

Nucleotide polymorphisms in STR markers. The Ibis approach to STR analysis measures the base composition of STR PCR products as well as the length, so an unambiguous allele call that is compatible with the current CODIS database is inherently achieved. The base composition of the product can reveal when there are polymorphisms within same-length alleles, essentially expanding the allele diversity of the existing marker set without the addition of new loci. What this means is that the Ibis assay can afford additional information from an STR analysis while remaining compatible with the vast data set already characterized in the CODIS database. We proposed to analyze the 13 CODIS loci for multiple members of the major Caucasian, African American and Hispanic populations to compile a catalog of allele frequencies including polymorphisms.

Nucleotide polymorphisms in STR markers for extended family analysis. The resolution of sequence polymorphisms within STR markers could be of aid in associating members of the same familial lineage. We had previously observed a substantial number of nucleotide polymorphisms within the CODIS STR loci in samples we had analyzed via ESI-MS. It is reasonable to assume that these polymorphisms would be relatively stable in human populations relative to the overall length of alleles, based upon expected base substitution rates vs. replication slippage rates in STR loci (potentially leading to addition or loss of repeat units). For example, estimates of point mutation rates in genes of humans and other mammals have been estimated at ca. $2x10^{-8}$ per nucleotide per generation, with hot-spot rates of $5x10^{-7}$ per nucleotide per generation or greater reportedly rare[1, 2]. This would suggest a possible SNP rate of up to $3x10^{-6}$ to $8x10^{-5}$ per generation for a 150 bp amplicon. Length-varying mutational rates in human STR loci (gain or loss of a repeat unit) have been estimated between ca. $5x10^{-4}$ per to $1x10^{-3}$ per generation[3, 4]. In informatics-based reports comparing predicted replication slippage rates to sequence polymorphism rates in STR loci, base

substitution rates were predicted to be anywhere from 10 to 1000-fold lower than repeat slippage rates, which is consistent with independent predictions and measurements of the two types of events[5-7].

In an analysis of two samples that may or may not be on the same direct lineage, or may or may not share ancestry, the presence of identical polymorphisms in same-length alleles could lend credence to analyses in support of shared ancestry. Because the SNP is likely to be a stable event relative to allele length, it is much less likely to have arisen through *de novo* mutation. Also, through analysis of Y-STR allele mutation rates in 4999 father/son pairs compared to published mutation rates for autosomal STR loci, it has been suggested that the mechanism of allele mutation generally does not involve recombination[8]. This supports the notion that nucleotide polymorphisms will be relatively stable and be faithfully passed from generation to generation. Also, in five of the CODIS loci (D3S1358, vWA, D13S317, D21S11 and D5S8181), we have observed that >20% of the alleles we have analyzed by ESI-MS have had polymorphisms relative to the same-length reference allele (see preliminary studies). In all cases, we have also observed the reference allele in other individuals, dispelling the notion that the reference allele used was simply a rare anomaly.

Y-chromosome markers. Genetic markers located on the Y-chromosome have recently found widespread application in the forensics community[9]. Y-chromosome markers, particularly Y-STR markers, have become a valuable tool in the analysis of evidence from sexual assault cases[10-17]. The benefit in Y-chromosomal marker in a mixed male/female sample is that the male contribution (generally the perpetrator) may be at a low level relative to the female contribution (generally the victim). Since the goal is to identify the perpetrator, the use of Y-chromosomal markers is a valuable asset because male DNA can be amplified specifically from a heavy background of female DNA. Y-chromosome markers also have found applications in paternity testing, missing persons investigations, and familial / genealogy analysis[18-22]. As an example of the value in long-range lineage studies, a direct male descendant and a male ancestor 10 generations removed would be expected to share only about a millionth of their DNA, whereas the Y-chromosome would most likely be identical in any given locus analyzed[18].

In collaboration with the FBI and AFDIL, and in part during phase I of this effort, we have developed a mitochondrial DNA (mtDNA) profiling assay based upon ESI-MS analysis of multiple PCR products. Mitochondrial DNA reflects only the maternal lineage of a family line and is often useful in kinship / extended family or missing persons analysis, as well as being a last resort method for typing old, damaged or limited DNA that is not amenable to autosomal analysis. Like mtDNA, a Y-marker profile is essentially a “haplotype” because there is only one copy per individual and there is essentially no recombination possible within the markers used in forensics applications, as markers are chosen that do not also exist on the X-chromosome[23]. The development of ESI-MS assays for Y-chromosome markers will complete a basic forensic genotyping platform based on ESI-MS where autosomal markers, Y-

chromosome markers and mtDNA profiling could be run on a single platform in an automated run mode.

Population statistics for polymorphisms in Y-STR markers. Just as in autosomal STR loci, core SWGDAM-recommended Y-STR loci used currently in forensic analyses are likely to have untapped alleles that differ only in sequence polymorphisms but have the same length. The ESI-MS approach to Y-STR analysis would inherently reveal these alleles and we proposed to analyze a population set of samples from the three major U.S. populations (Caucasian, African American and Hispanic) to compile nucleotide-polymorphic Y-STR allele frequencies for the core SWGDAM Y-STR locus set. The addition of relatively stable SNP variations with the highly-polymorphic length variation in Y-STRs will lend substance to an inclusion between a sample and a profile from an individual or relative.

Nucleotide polymorphisms in Y-STR markers for paternal lineage / familial linkage analysis. Because forensically-valuable Y-chromosome markers do not experience recombination during meiosis, and there is only one Y-chromosome to choose from during gametogenesis, all analyzed markers on the Y-chromosome for a given sample must be treated as a single locus. The product rule does not apply to assess expected profile frequencies from a population. In fact, the entire Y-chromosome is really a single a locus, since it passes to the next generation as a single unit (with the exception of small portions that recombine with the X-chromosome, and these regions are not used in a typical Y-marker panel)[23, 24]. An effort should be made to utilize a maximum of information from each analyzed marker. The ability to resolve polymorphisms between Y-STR alleles could aid in paternal lineage analyses, especially when only a few markers are amplifiable (for example, from a disaster victim or dead body discovered after a long period of time).

Literature citations and review

There has been substantial effort in the forensics community to improve the performance and sensitivity of STR analysis. A considerable amount of attention has been given to shortening amplified products, commonly referred to as "Mini-STRs" in order to improve the sensitivity and success rate upon limited and degraded DNA samples[25-30]. The Ibis technology is actually complementary to this methodology and utilizes the same approach. Ibis primer pairs for STR analysis are placed as close to the repeat as possible, primarily to increase the resolution obtained during mass spectrometry analysis. This approach has the parallel benefit of shorter products that are more likely to work upon degraded templates. In preliminary studies, we had obtained perfect concordance with MiniFiler[30] results on 95 population reference samples provided by NIST (see the final report to phase I of this effort, attached in "Additional documents"). The primary advantages provided by the Ibis approach are 1.) Nucleotide polymorphisms are inherently revealed during analysis, 2.) Prior information about nucleotide polymorphisms is not required (neither the prior characterization nor prior localization of a SNP within a PCR product are required for it to be correctly identified), 3.) No allelic ladder is required for any locus analyzed, 4.) no primer labels are required (products are analyzed directly in the mass spectrometer), and 5.) Alleles of different loci can cross in length, and same-length alleles of one locus can be differentiated if they differ in base composition. Moreover, if a novel allele with a length previously not seen is detected, a base composition can be derived directly and a very reasonable hypothesis can be made immediately about the allele structure (although sequencing is still required to ultimately confirm the sequence structure).

Other approaches to improving or enhancing current analysis systems involve adding more loci[27], the use of amplicon sequencing[31], analysis of SNP panels via "minisequencing," "SNapShot," pyrosequencing, array-based strategies (e.g. luminex), or MALDI-TOF-MS[32-37]. While each of these methodologies certainly has merit, our goal here was to provide a technology that adds value to the existing methodology while maintaining compatibility with the >5,000,000 STR profiles already deposited in the CODIS system. While allele sequencing is capable of revealing all microvariation within STR alleles, sequencing of autosomal alleles requires prior separation of the two alleles, and even sequencing of Y-chromosomal STRs requires considerable biochemical manipulation. SNP assays generally require precise knowledge of the location of each SNP, whereas ESI-MS analysis of existing STR loci reveals nucleotide polymorphisms with no prior characterization of their locations.

Previous Approaches to MS-based Forensic DNA Analysis. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI TOF MS) has been previously employed by others to analyze STR, SNP, and Y-chromosome markers [38-42]. To obtain routinely the necessary mass accuracy and resolution using MALDI TOF MS, the amplicon size must be less than 100 bp, which often requires strategies such as enzymatic digestion and nested linear amplification[43, 44]. In the MALDI approach, PCR

amplicons must be thoroughly desalted and co-crystallized with a suitable matrix prior to mass spectrometric analysis. The size reduction schemes and clean-up schemes employed for STR and SNP analyses in the cited reports resulted in the mass spectrometric analysis of only one strand of the PCR amplicon[38, 41, 42]. By measuring the mass of only one strand of the amplicon, an unambiguous base composition cannot be determined and only the length of the allele is obtained. Even with the size reduction schemes, mass measurement errors of 12 to 60 Daltons (Da) are observed for products in the size range 15000 to 25000 Da [41]. This corresponds to mass measurement errors of the 800 to 2400 ppm. Because of mass accuracy limitations and mass resolution typical of MALDI, multiplexing of STRs is difficult and not routine, although in one published report three STR loci were successfully multiplexed[38]. The issue of allelic balance has not been addressed for MALDI-TOF-MS based assays.

The mass accuracy and resolution obtained with electrospray ionization (ESI) TOF MS is significantly improved relative to MALDI TOF MS. With amplicons in the 120-150 bp range, we have been able to obtain mass measurement errors of less than 20 ppm routinely with ESI[45, 46]. This allows multiplexing of STR loci. We have successfully multiplexed 6 STR loci in multiple combinations, and are currently moving forward with an assay based upon multiple three-plex reactions. We have also developed a highly automated PCR cleanup scheme that is compatible with ESI TOF. Unlike the MALDI TOF examples above, both strands of the amplicon are observed when using ESI TOF. Observation of both strands of the amplicon allows unambiguous base composition determination and confirmation of allele calls as well as the ability to determine SNPs present in alleles (see preliminary results). With automation, ESI TOF systems can analyze a well every 56 seconds. High throughput capacities of 1536 wells/day can be obtained. Thus, ESI TOF shows great promise for the analysis of PCR amplicons.

Rationale for the research

In this project we proposed to build upon work initiated in the phase I grant #2006-DN-BX-K011, born in part out of collaborations between Ibis and both the DNA Forensics Division of the FBI (Dr. Bruce Budowle) and the Armed Forces Institute of Pathology DNA Identification Laboratory (Colonel Brion Smith, DDS, now retired) in which we have made advances developing a next generation DNA forensics platform based on high throughput electrospray ionization mass spectrometry (ESI-MS). The approach is based on using ESI-MS to “weigh” DNA forensic markers with enough accuracy to yield product base compositions (number of A’s, G’s, C’s and T’s). Importantly, these base composition profiles can be referenced to existing forensics databases derived from mtDNA sequence, STR, or Y-STR profiles.

We had done preliminary blinded validation studies with this approach in collaboration with both the FBI and AFIP/AFDIL to evaluate the platform for both STR and mtDNA typing. Importantly, the same platform is used for both types of analyses and in both approaches the MS offers distinct advantages over the conventional approach. Because base compositions are used to derive specific alleles, the MS-

based method picks up SNPs within STR regions that go undetected by conventional electrophoretic analyses. For example, all “allele type 11” for the D13S317 marker are not equivalent; some contain an A to T SNP which distinguish them from individuals containing the “normal” allele type 11. Similarly, individuals which are typed as homozygous for this allele may in fact be heterozygotes containing alleles 11 and 11 (A→T). During our phase I effort, we observed that 100% of 95 population reference samples obtained from NIST had at least one nucleotide-polymorphic allele within the core 13 CODIS STR loci.

We proposed to further develop the ESI-MS approach to STR analysis and expand the approach to the analysis of Y-chromosome STRs. We proposed to analyze sets of samples to compile nucleotide-polymorphic allele frequencies in the core CODIS STR loci and the standard forensic Y-STR loci. We also proposed to analyze samples linked by extended family relationships to verify the faithful transmission of polymorphic alleles and their utility in adding resolution to current STR typing assays. We believe that this approach has the potential to revolutionize the way DNA forensics is practiced. Further development and validation of this platform will yield a system that provides increased discriminatory power while offering the cost and throughput advantages inherent to a fully automated platform.

Methods, results and conclusions

This effort involved the development of a new technology for analysis of forensic markers and has culminated in the production of a manufactured STR kit that is currently undergoing the commercialization process, as well as production of a preliminary Y-STR research-grade kit. Due to the nature of the development effort, it is more straightforward to present methods, results and conclusions together, organized by specific aims as outlined in the original proposal. To provide a context base, background information is provided in each section. This information is marked with the line **"*Background Material"***. New work in each section is delineated by the line **"*New work under the current award"***.

SUMMARY OF SPECIFIC AIMS

The following objectives were proposed in Invited Application #2008-90554-CA-DN that led to this contract award:

Specific Aim 1 Complete the implementation of a new robust STR panel on the Ibis T5000 platform.
Specific Aim 2: Develop an ESI-MS assay for the SWGDAM-recommended Y-STR markers.

2.1 Development of a multiplex Y-STR assay
2.2 Sensitivity
2.3 Species specificity
2.4 Reproducibility and accuracy
2.5 Testing against a panel of samples / population studies

Specific Aim 3: Characterize polymorphisms in core autosomal STR and Y-STR markers
Specific Aim 4: Analysis of extended family samples.
Specific Aim 5: Continued development of transferable analysis software with an intuitive user interface

5.1 Complete the STR assay data processing automation
5.2 Refine the STR analysis interface

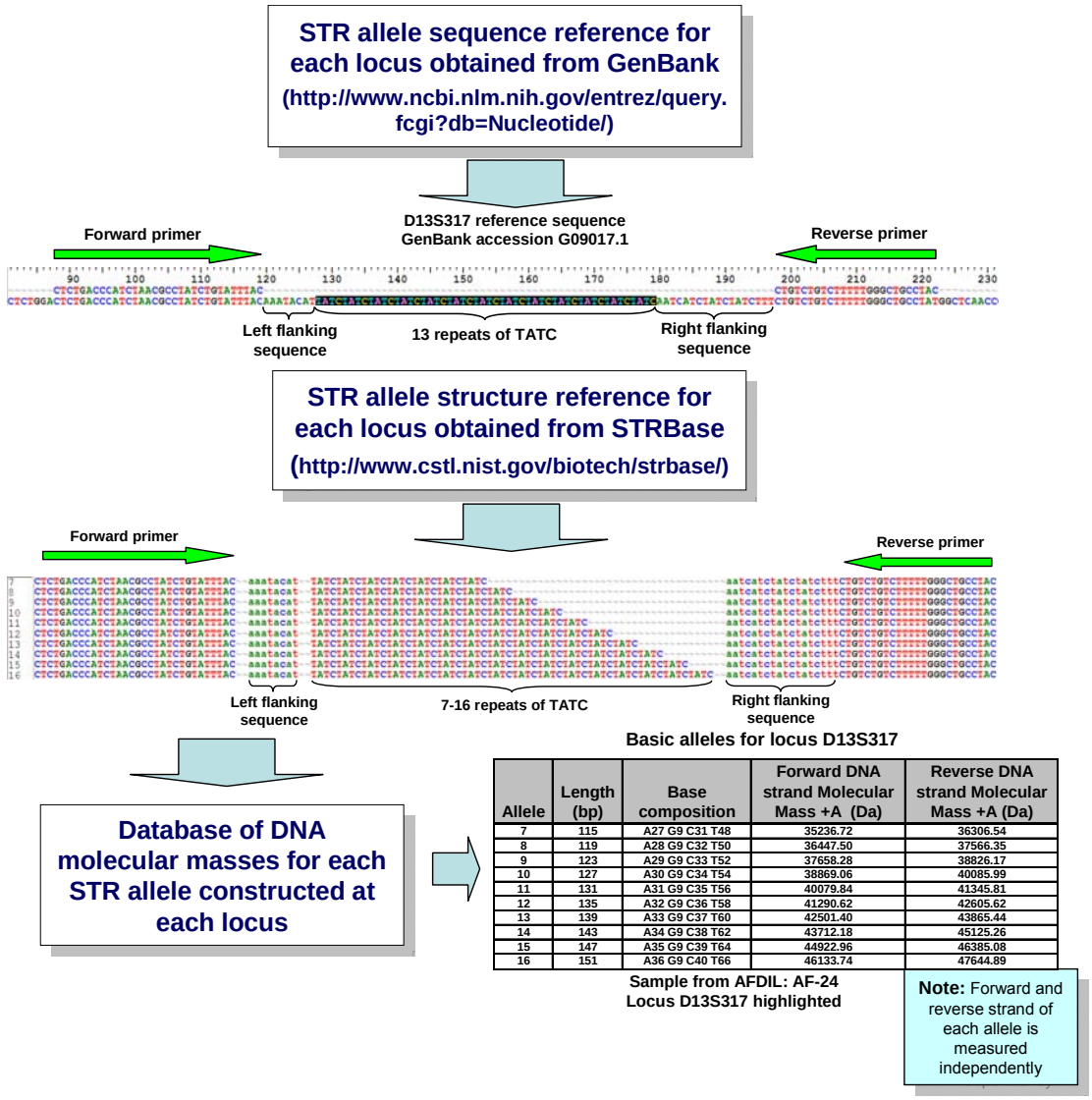
Specific Aim 1: Complete the implementation of a new robust STR panel on the Ibis T5000 platform.

Background Material:

The principle elements of our STR assay are the measurement of PCR product masses via Electrospray-ionization time-of-flight mass spectrometry (ESI-TOF-MS), determination of product base compositions from their masses[45], and the association of the product base compositions to a database of alleles for each locus. The mass of a PCR product is an inherent property of the product that does not change according to assay conditions. Unlike measurement of product mobility in a gel, therefore, the measurement of PCR product masses does not require an allelic ladder to correctly assign a product to the allele it represents. The mass of a given allele generated with a

specific primer pair is static and precise. We populate a database of all known alleles based upon a reference sequence and the published allele structures for each of the loci (obtained from STRBase[47]). The basic outline of generation and use of the database in this assay is outlined in Figure 1. Using accurate mass measurements, we can determine when an allele has a polymorphism within the amplified region relative to the reference allele because the polymorphism changes the base composition of the PCR product. The following section reviews the general description of the Ibis STR assay and briefly overviews progress from the original phase I effort and the first period of the current phase.

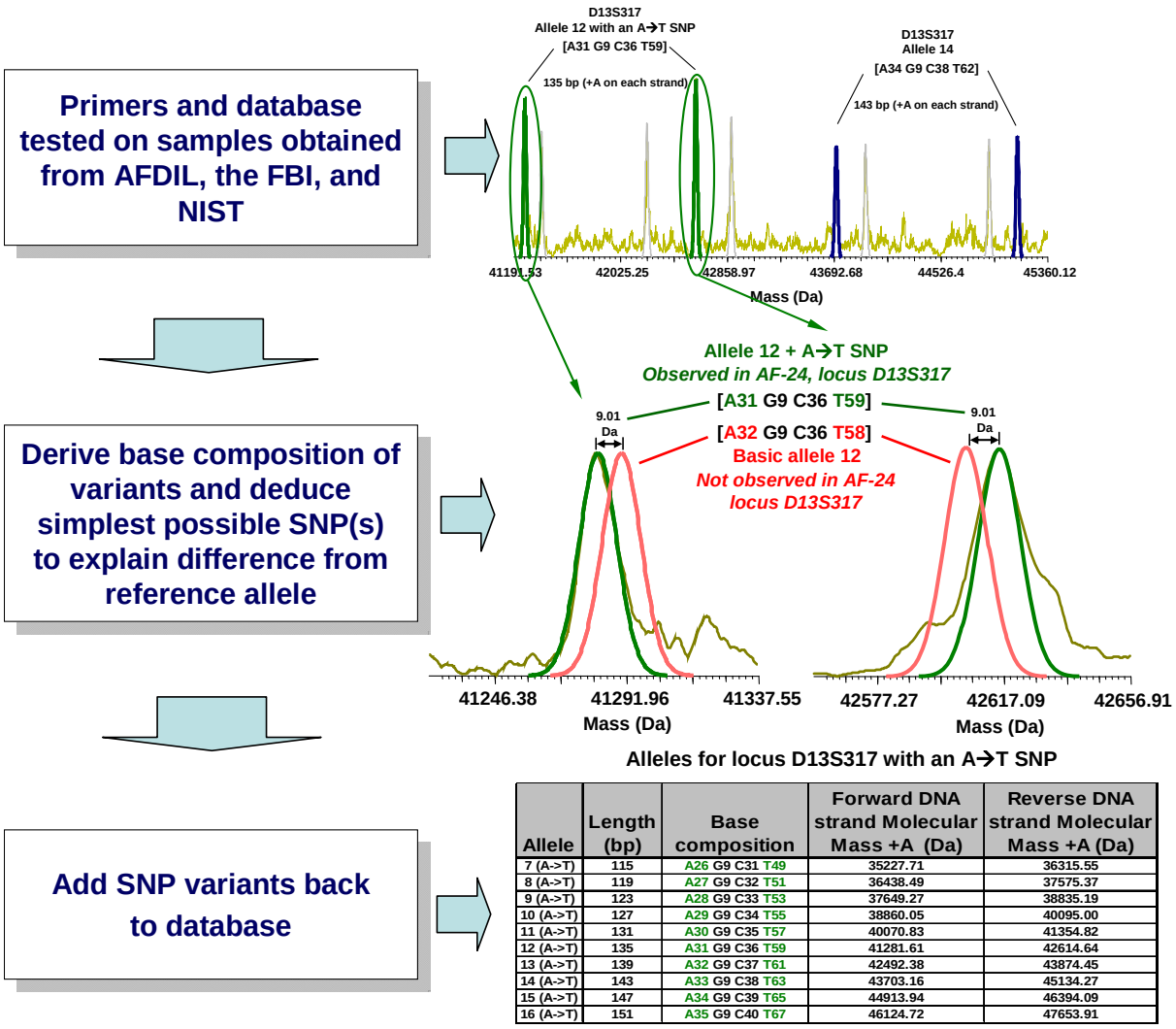
A.



Allele	Length (bp)	Base composition	Forward DNA strand Molecular Mass +A (Da)	Reverse DNA strand Molecular Mass +A (Da)
7	115	A27 G9 C31 T48	35236.72	36306.54
8	119	A28 G9 C32 T50	36447.50	37566.35
9	123	A29 G9 C33 T52	37658.28	38826.17
10	127	A30 G9 C34 T54	38869.06	40085.99
11	131	A31 G9 C35 T56	40079.84	41345.81
12	135	A32 G9 C36 T58	41290.62	42605.62
13	139	A33 G9 C37 T60	42501.40	43865.44
14	143	A34 G9 C38 T62	43712.18	45125.26
15	147	A35 G9 C39 T64	44922.96	46385.08
16	151	A36 G9 C40 T66	46133.74	47644.89

Figure 1. Panel A. The process of generating reference allele entries for an STR allele database is outlined above using D13S317 as an example.

B.



Allele	Length (bp)	Base composition	Forward DNA strand Molecular Mass +A (Da)	Reverse DNA strand Molecular Mass +A (Da)
7 (A->T)	115	A26 G9 C31 T49	35227.71	36315.55
8 (A->T)	119	A27 G9 C32 T51	36438.49	37575.37
9 (A->T)	123	A28 G9 C33 T53	37649.27	38835.19
10 (A->T)	127	A29 G9 C34 T55	38860.05	40095.00
11 (A->T)	131	A30 G9 C35 T57	40070.83	41354.82
12 (A->T)	135	A31 G9 C36 T59	41281.61	42614.64
13 (A->T)	139	A32 G9 C37 T61	42492.38	43874.45
14 (A->T)	143	A33 G9 C38 T63	43703.16	45134.27
15 (A->T)	147	A34 G9 C39 T65	44913.94	46394.09
16 (A->T)	151	A35 G9 C40 T67	46124.72	47653.91

Figure 1. Panel B. The use of an allele database in the absence of an allelic ladder. Correct allele assignments can be made by the direct measurement of product masses and the subsequent calculation of product base compositions. A sequence polymorphism in the allele relative to the reference allele results in shifted masses of both the forward and reverse strands. Calculation of the product base composition reveals the polymorphism(s). Polymorphic alleles can then be added back to the database. The location of the polymorphism remains unknown unless the allele is sequenced. Also, if two cancelling polymorphisms are present (e.g. and A→G SNP and a G→A SNP within the same amplicon), the ESI-TOF-MS assay will not register a polymorphism. This is expected to be quite rare in STR alleles, however.

As part of phase I award 2006-DN-BX-K011, a multiplex STR assay that covers the 13 core CODIS loci and the amelogenin sex marker was developed[48]. The basic layout of this assay at the close of the phase I award is shown in Figure 2.

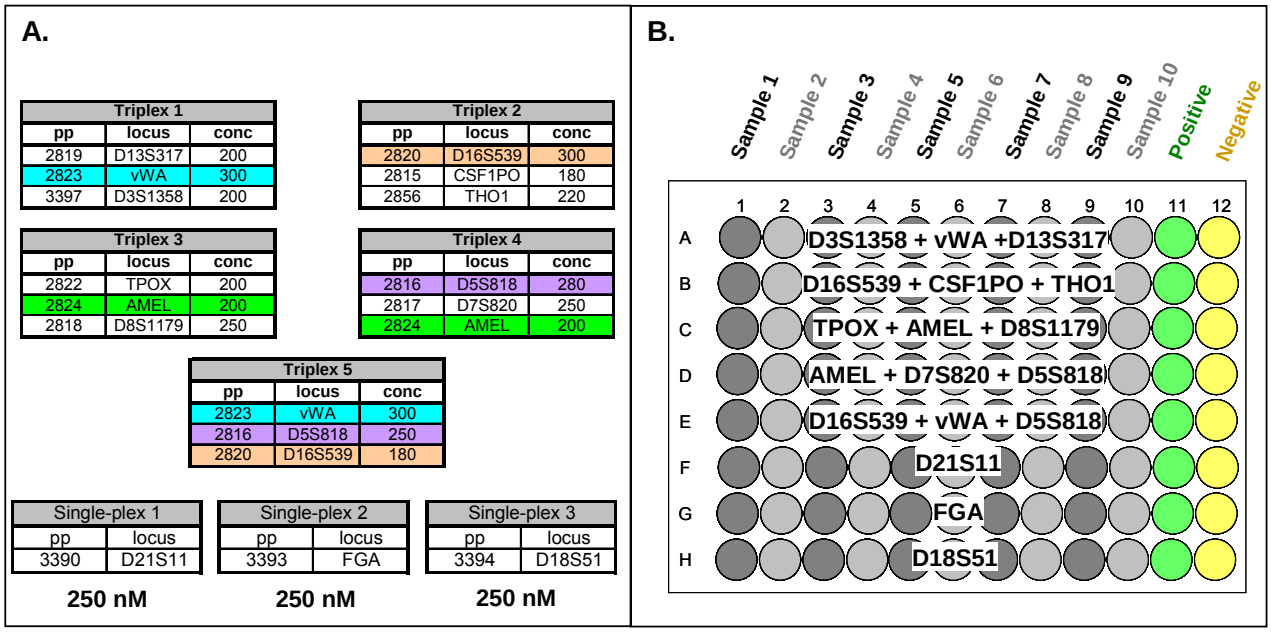


Figure 2. Assay layout for a 14-locus ESI-TOF-MS STR assay. Each sample is distributed across 8 wells in one column each of a 96-well assay plate. A standard layout could consist of 10 samples, one positive control and one negative control on an assay plate. A.) Primer pair groupings for each reaction set. There are eight reaction sets (five tri-plexes and three single-plexes). Primer pair concentrations in tri-plexed reactions are adjusted to achieve acceptable inter-locus product balance. Working concentrations of primers in each primer pair are shown in the right column of each table. All single-plex reactions are performed using 250 nM of each primer. B.) Proposed layout of a standardized assay plate for a 14-locus STR assay.

Although the assay generated results concordant with existing databases, revealed polymorphisms in alleles, and was sensitive down to ~100-200 pg per reaction, the current assay would benefit from some modification to increase the dynamic range possible for detection of same-length alleles from a mixture containing unequal contributor inputs. The primer pairs and PCR conditions favored complete non-templated adenylation of PCR products, as in conventional STR typing. The resolution of the mass spectrometer, however, reveals that adenylation by *Taq* polymerase may not always be 100% specific. For example, Figure 3 shows that a small amount of 'C', 'T' or 'G' a sometimes added to the PCR product, rather than the expected 'A'. The ability to detect mixtures of alleles with different repeat counts (as in conventional typing) is not affected by a low level of non-specific nucleotide addition. However, this assay is capable of resolving mixed alleles even of the same length, as long as they

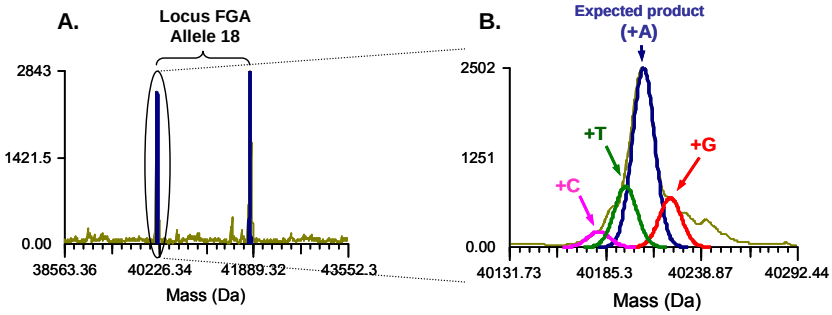


Figure 3. Final adenylation of PCR products is not 100% specific. A). PCR product assignments are clear and solid from mass spectrometry data. Example = locus FGA, allele 18. B.) Detail view of the forward strand. There is a small amount of apparent +G, +C and +T products in addition to the expected +A product, presumably caused by incomplete specificity of *Taq* polymerase terminal transferase activity.

differ by a nucleotide polymorphism(s). The dynamic range for equal-length alleles would thus be limited by the level of non-specific terminal transferase activity from *Taq* polymerase. For example, in the allele shown in Figure 3, an allele 18 (A→G) would fall in the same space as the allele 18 with a 'G' added instead of an 'A' by Taq polymerase terminal transferase activity. With sufficient single-contributor template, this should not diminish the ability to call an 18, 18 (A→G) because the levels of the two alleles should be approximately equal and the mass signals resolve clearly. However, if more than one contributor sample is present, or if the template level is low enough to cause stochastic sampling effects, it could be difficult to distinguish an artifact from a true contributor allele.

New work under the current award:

Preliminary assay redesign work

In an attempt to reduce biochemical artifacts imposed by non-specific nucleotide transferase activity, a strategy was implemented to minimize *Taq* polymerase's tendency to add an adenosine to the 3′ end of PCR products. The 14 primer pairs referred to in Figure 2 (sequences available in Hofstadler and Hall, 2008[48]) were modified to initiate with one or more thymidine residues at the 5′ end. This ensures that the both strands of the PCR product will end on a 3′ adenosine. *Taq* polymerase has been shown to have greatly diminished activity adding a base to an existing 3′ adenosine[49-52]. Initial primer pair choices are shown in Table 1. Primers were tested upon positive control DNA sample N31774 at 1 ng template per 40 μl reaction in 10 mM Tris-Cl, 75 mM KCl, 400 mM betaine, 1.5 mM $MgCl_2$, 200 uM each dNTP (BioLine), 5 U/reaction AmpliTaq Gold, and the primer pair concentrations used for preceding primer pairs from phase I of this effort (Table 2), or in mixes all containing 250 nM each primer pair. Thermocycling consisted of 95 °C, 10 min, 40 cycles of [95 °C, 20 sec, 56 °C 1.5 min, 72 °C, 45 sec], 72 °C, 4 min, 4 °C hold. For each primer pair concentration mix, tests were run in triplicate. For positive control template N31774, all allele calls were assigned correctly in all reactions. There was a general improvement in the purity of

Table 1. STR primer pairs redesigned to minimize PCR product end adenylation by *Taq* polymerase.

Locus	Ibis PP Number	pp name	Primer sequences
CSF1PO	3882	CSF1PO_U63963_11909_12040	TTGGCATGAAGATATTAACAGTAACTGCCTTCATA TTGTGTCAGACCCTGTTCTAAGTACTTCCT
D3S1358	3883	D3S_NT086638_5793095_5793208	TTGAAATCAACAGAGGCTTGCATGTAT TTGACAGAGCAAGACCCTGTCTCAT
D5S818	3884	D5S818_G08446_68_224	TTGGGTGATTTTCCTCTTTGGTATCCTTATGTAAT TTGCCAATCATAGCCACAGTTTACAACATTTGTA
D7S820	3885	D7S820_G08616_91_231	TTGGGAACACTTGTCATAGTTTAGAACGAACTA TTCCCGGAATGTTTACTATAGACTATTTAGTGAGAT
D8S1179	3886	D8S1179_G08710_12_130	TTGGGGTTTTGTATTTCATGTGTACATTCGTATC TTGGGTACCTATCCTGTAGATTATTTTCACTGTGG
D13S317	3887	D13S317_G09017_86_224	TTCTCTGACCCATCTAACGCCTATCTGTATTTAC TTGTAGGCAGCCCAAAAAGACAGACAG
D16S539	3888	D16S539_G07925_230_353	TTGCTCTTCCTCTTCCCTAGATCAATACAGACA TTGCTACCATCCATCTCTGTTTTGTCTTTCAATG
D18S51	3889	D18S51_AP001534_85738_85902	TTGATGTCTTACAATAACAGTTGCTACTATTTCT TTCTGAGTGACAAATTGAGACCTTGTC
D21S11	3890	D21S11_M84567_135_290	TTCCCCAAGTGAATTGCCTTCTA TTGGTAGATAGACTGGATAGATAGACGATAGA
FGA	3891	FGA_M64982_2865_3012	TTCCCAATTAGGCATATTTACAAGCTAGTT TTGTCTGTAATTGCCAGCAAAAAAGAAA
THO1	3892	THO1_D00269_1105_1240	TTGGAAATCAAAGGGTATCTGGGCTCTGG TTCGCTGGTCACAGGGAACACAGAC
TPOX	3893	TPOX_M68651_1839_1949	TTGGCACAGAACAGGCACTTAGGGA TTGGTGTCCTTGTCAGCGTTTATTTGCC
vWA	3894	VWA_M25858_1649_1791	TTGGGGAGAATAATCAGTATGTGACTTGGATTG TTGGGTGATAAATACATAGGATGGATGGATAGATGG
AMEL	3895	AMEL_M55418_284_396	TTGCCCTGGGCTCTGTAAAGAATAGTG TTGCATCAGAGCTTAAACTGGGAAGCTG

observed peaks compared to those observed when forcing full adenylation. Figure 4 demonstrates this with a comparison of the same allele from the same sample with

Table 2. Initial testing concentrations for 1st round STR primer modifications. The three primer pairs not shown (3890, 3891 and 3889, see Table 1) were used at 250 nM each in single-plex reactions.

Mix	pp	Locus	conc (nM)
A	3887	D13S317	200
	3894	vWA	300
	3883	D3S1358	200
B	3888	D16S539	300
	3882	CSF1PO	180
	3892	THO1	220
C	3893	TPOX	200
	3895	AMEL	200
	3886	D8S1179	250
D	3884	D5S818	280
	3885	D7S820	250
	3895	AMEL	200
E	3894	vWA	300
	3884	D5S818	250
	3888	D16S539	180

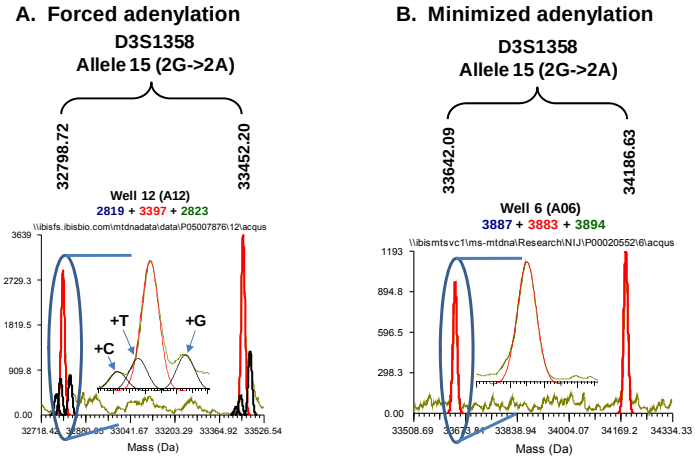


Figure 4. Comparison of peak purity when forcing full adenylation of products or minimizing product adenylation. Allele 15 (with 2G→2A SNPs) from locus D3S1358 from sample N31774 run with forced adenylation using the protocol described in Hofstadler and Hall, 2008[48] (panel A), or the same allele when sample N31774 was run with new primer pairs in Table 1 using 1.5 mM $MgCl_2$. Note that product masses are not identical in panels A and B because of nucleotide differences in the 5′ ends of the primers.

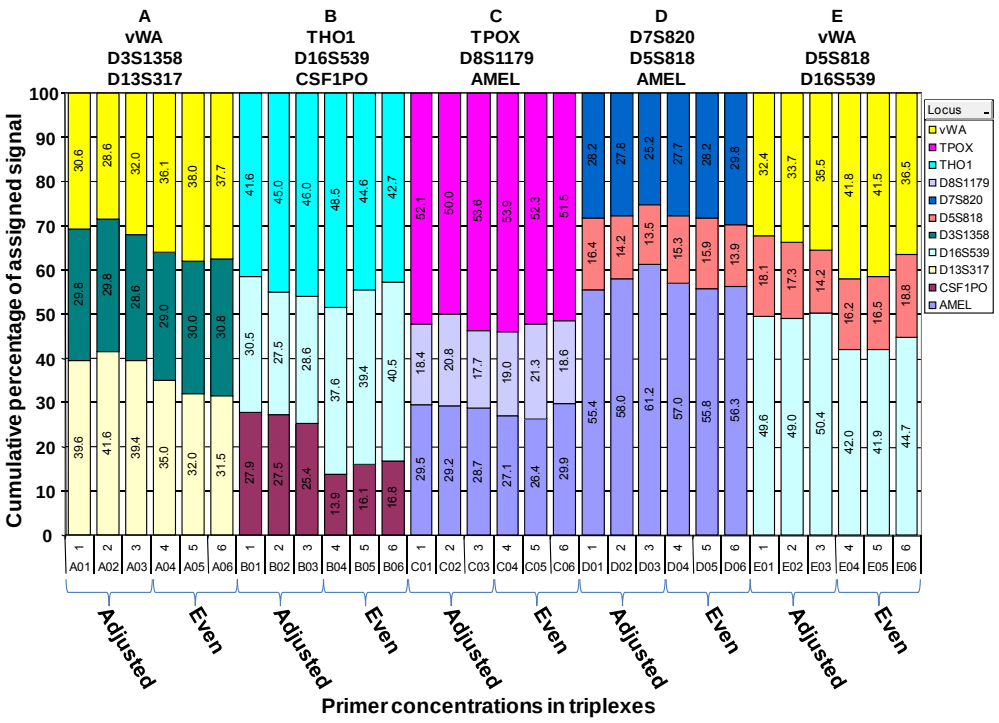


Figure 5. Inter-locus balance of redesigned primer pairs in initial tests against positive control templates. Primer pair concentrations were either adjusted to the values shown in Table 2 ("Adjusted"), or run at 250 nM each ("Even"), as indicated above. Loci included in each triplex are indicated above the chart. Each triplex was run in triplicate for each primer pair mix. Adjusted primer concentrations produced only marginally better balance in triplexes than even primer pair concentrations.

forced adenylation (panel A) or minimized adenylation using the new primer pairs (panel B). However, inter-allelic balance within triplexes was suboptimal in both mixing scenarios (Figure 5).

A.

Mix	pp	locus	conc
A	3887	D13S317	250
	3894	vWA	325.0
	3883	D3S1358	325.0
B	3892	THO1	225.0
	3888	D16S539	325.0
	3882	CSF1PO	350.0
C	3893	TPOX	165
	3895	AMEL	285.0
	3886	D8S1179	450.0
D	3895	AMEL	130.0
	3885	D7S820	270.0
	3884	D5S818	500.0
E	3888	D16S539	170.0
	3894	vWA	240.0
	3884	D5S818	490.0

B.

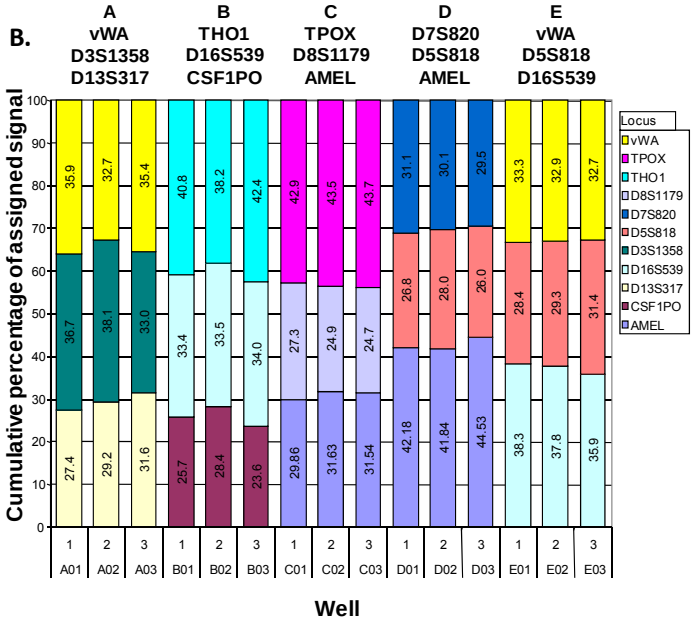


Figure 6. Inter-locus balance of redesigned primer pair tests against positive control templates after adjusting relative primer pair concentrations. Primer pair concentrations were adjusted to the values shown panel A. Each triplex was run in triplicate. Adjusted primer concentrations produced modestly better balance in triplexes than previous primer pair concentrations (panel B). Loci included in each triplex are indicated above the chart.

Primer pair concentrations were adjusted according to their relative signal outputs represented in Figure 5. Also, with the cycling parameters described above and the primer pairs shown in Table 1, there was considerable adenylation in some of the larger products (e.g., FGA, D21S112 and D18S51, not shown). Cycling was thus modified to reduce the extension time to 25 sec and the final 72 °C step to 2 min. These modifications made another modest improvement in overall inter-locus product balance (Figure 6). Several primer pairs did not produce evenly-balanced forward and reverse strands within individual PCR products. Figure 7 shows the average inter-product ratios for the 14 loci using the primer pairs shown in Figure 1. Primer pairs for three loci (D13S317, D16S539 and FGA) had higher:lower intra-product strand abundance ratios exceeding 1.5 (Figure 7) and alternative primer designs were considered and tested for them.

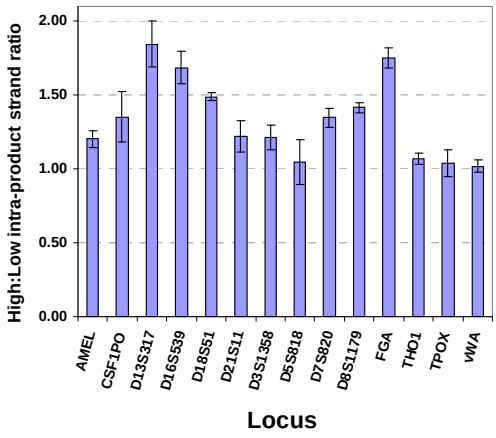


Figure 7. Average intra-product strand ratios (± stdev) for STR primer pairs shown in Table 1. Primer pairs for D13S317, D16S539 and FGA were redesigned.

Mass tagging strategy

We have implemented a mass-tagging strategy that affords great accuracy in base composition assignments (Figure 8). We have applied this strategy to our STR analyses to unambiguously assign the identity of nucleotide polymorphisms observed in STR analyses. Because an, 'A' weighs ~313.2 Da and a 'G' weighs ~329.2 Da, a base switch from an 'A' to a 'G' results in a mass shift of ~16 Da, which is very easy to measure in the mass spectrometer. However, a 'C' weighs ~289.2 Da and a 'T' weighs ~ 304.2 Da, meaning that a base switch from 'C' to 'T' results in a mass shift of ~15 Da, which is only 1 Da different than an A→G switch. Although we use base composition complementarity to assign double-stranded products, an A→G on one strand is a T→C on the complementary strand, and a C→T on one strand is a G→A on the complementary strand, thus the complementary strands resulting from an A→G and a C→T are also 1 Da different from each other. As shown in Figure 8, the incorporation of a ^{13}C-enriched dGTP in place of normal dGTP changes the mass of one nucleotide (G) by making it ~10 Da heavier while not altering the other nucleotide masses. This mass shift results in widely-separated mass shifts for all possible combinations of base changes from any starting composition where A, G, C, T counts are each within ±10 of the starting base count. The ^{13}C-dGTP mass-tagging strategy has been fully incorporated into all STR work from PCR reaction composition to data processing and software-aided interpretation.

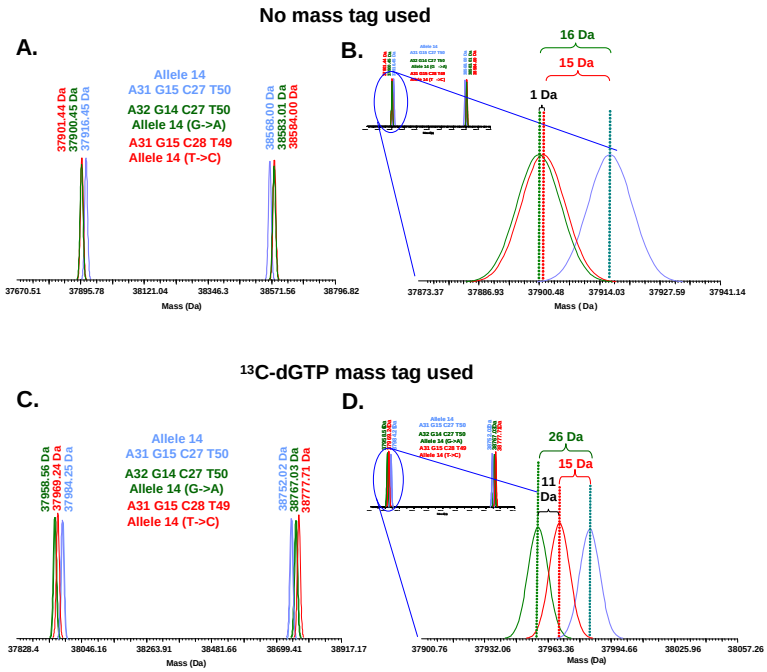


Figure 8. Use of a mass tag to make an unambiguous SNP assignment in a PCR amplicon. The example above shows locus D8S1179, allele 14, amplified with Ibis primer pair 2818. A.) Amplified with natural dNTPs, a G→A and a T→C variant produce amplicons very close in mass (about 1 Da difference). B.) Zoomed-in view of forward strand masses for allele 14 base, with a G→A, and with a T→C SNP. There is an unambiguous detection of a SNP from the base allele 14, but only a 1 Da difference between masses for G→A and T→C products, making the SNP potentially ambiguous between two possibilities. C.) When amplified with ^{13}C-enriched dGTP in place of dGTP, a G→A and a T→C variant from allele 14 produce amplicons separated by nearly 11 Da, which allows unambiguous assignment of each SNP variant. D.) Zoomed-in view of the forward strand masses for each of the three PCR products amplified with ^{13}C-enriched dGTP. There is an unambiguous detection of a SNP from the base allele 14 product, and an unambiguous assignment of the base switch involved in the SNP. The basic allele 14 product is separated from the G→A SNP by ~26 Da and from the T→C SNP by ~15 Da. The two SNP variants are separated by ~11 Da.

Primer pair modifications forced by assay redesign

The implementation of the mass-tagging strategy forced the redesign of one of the STR primer pairs shown in Table 1. The primer pairs were designed with the forward and reverse product masses in mind, but the mass-tagging shifts the masses only according to the number of G residues in each strand. Thus, forward and reverse strands can shift by different amounts for each of the primer pairs. For D5S818, this caused the forward strand of an allele to collide in mass with the reverse strand of the next allele up if it had a G→T SNP in it (see Figure 9. A, showing data generated for sample N31774 containing genotype 11, 12 (G→T) for D5S818). A redesign of the D5S818 primer pair moved the strand masses away from each other (Figure 9. B). In addition to the four loci mentioned above, altered primer pairs were also investigated and ultimately switched for D21S11 and D18S51, primarily to reduce non-templated adenosine addition and improve intra-product strand balance (data not shown). The primer pairs and concentrations in the assay panel at this stage of development is shown in Table 3.

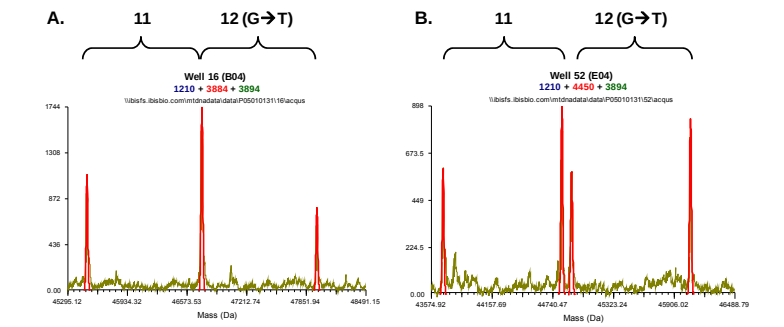


Figure 9. Redesign of D5S818 primer pair 3884 to separate masses of strands from products of alleles 11 and 12 (G→T). In panel A, primer pair 3884 was run with the 13C-dGTP mass tag that will be used in production moving forward. The forward strand of allele 11 (the heavier strand in this case) has a mass that is only ~4 Da different than the reverse mass for allele 12 (G→T), although these masses separated without the mass tag. In panel B, the redesigned primer pair 4450 produces masses that separate from each other.

Table 3. Primer pairs used in preliminary state of a redesigned STR assay. Primer pairs and sequences are shown in panel A. Working concentrations of multiplexed primer pairs are shown in panel B. Primer pairs for FGA, D21S11 and D18S51, which are each run separately, were used at 250 nM each.

A.

Locus	Ibis PP Number	Primer pair name	Primer sequences
AMEL	3895	AMEL_M55418_284_396	TTGCCCTGGGCTCTGTAAAGAATAGTG TTGCATCAGAGCTTAAACTGGGAAGCTG
CSF1PO	3882	CSF1PO_U63963_11909_12040	TTGGCATGAAGATATTAACAGTAACTGCCTTCATA TTGTGTCAGACCCTGTTCTAAGTACTTCCT
D13S317	1216	D13S317_G09017_84_221	TGGACTCTGACCCATCTAACGCCTATC TAGGCAGCCCAAAAAGACAGACAGAA
D16S539	1210	D16S539_G07925_234_349	TCTTCCTCTTCCCTAGATCAATACAGACAG TACCATCCATCTCTGTTTTGTCTTTCAATG
D18S51	1205	D18S51_AP001534_85734_85901	TGTGGAGATGTCTTACAATAACAGTTGCTACTA TCTGAGTGACAAATTGAGACCTTGTCTC
D21S11	4451	D21S11_M84567_134_292	TTTTCCCAAGTGAATTGCCTTCTATC TTGAGGTAGATAGACTGGATAGATAGACGA
D3S1358	3883	D3S_NT086638_5793095_5793208	TTGAAATCAACAGAGGCTTGCATGTAT TTGACAGAGCAAGACCCTGTCTCAT
D5S818	4450	D5S818_G08446_71_221	TGTGATTTTCCTCTTTGGTATCCTTATGTAAT TCAATCATAGCCACAGTTTACAACATTTG
D7S820	3885	D7S820_G08616_91_231	TTGGGAACACTTGTCATAGTTTAGAACGAACTA TTCCCGGAATGTTTACTATAGACTATTTAGTGAGAT
D8S1179	3886	D8S1179_G08710_12_130	TTGGGGTTTTGTATTTCATGTGTACATTCGTATC TTGGGTACCTATCCTGTAGATTATTTTCACTGTGG
FGA	4448	FGA_M64982_2866_3019	TTGCCCTTAGGCATATTTACAAGCTAG TTGTGATTTGTCTGTAATTGCCAGCAA
THO1	3892	THO1_D00269_1105_1240	TTGGAAATCAAAGGGTATCTGGGCTCTGG TTCGCTGGTCACAGGGAACACAGAC
TPOX	3893	TPOX_M68651_1839_1949	TTGGCACAGAACAGGCACTTAGGGA TTGGTGTCCTTGTCAGCGTTTATTTGCC
vWA	3894	VWA_M25858_1649_1791	TTGGGGAGAATAATCAGTATGTGACTTGGATTG TTGGGTGATAAATACATAGGATGGATGGATAGATGG

B.

Mix	pp	Locus	conc (nM)
A	1216	D13S317	250
	3894	vWA	325.0
	3883	D3S1358	325.0
B	3892	THO1	225.0
	1210	D16S539	325.0
	3882	CSF1PO	350.0
C	3893	TPOX	200
	3895	AMEL	270.0
	3886	D8S1179	430.0
D	3895	AMEL	160.0
	3885	D7S820	280.0
	4450	D5S818	460.0
E	1210	D16S539	175.0
	3894	vWA	225.0
	4450	D5S818	500.0

Table 4. Comparison of 40 NIST population reference samples[53] run on new STR test plates to previously-generated profiles that were concordant with NIST results for basic allele calls[48]. Genotypes that were concordant with previous results are highlighted green. Genotypes that were not concordant are highlighted in Fuchsia. There were two discrepancies. Both discrepancies were an allele 9 that dropped out from locus D13S317. Allele sequencing was performed with the affected samples for D13S317, revealing an end mismatch on the 3' end of a primer (see explanation in text).

Population	Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA
African American	JT51471	X	10	12	9	16	28	15 (G->A)	11	8	12 (A->G)	28 (T->C)	6	8	16
		Y	13	---	13	---	33.2	16 (G->A)	---	10	13 (A->G)	31.2	7	---	---
African American	JT51499	X	11	11	11	13	30 (A->G)	15 (G->A)	9 (G->T)	10	14	22	7	8	16 (G->A)
		Y	12	---	12	16	---	18	12	11	14 (A->G)	26	9.3	---	18
African American	OT05888	X	10	11	10	16	31.2	15 (G->A)	12 (G->T)	9	13 (A->G)	19	7	8	16
		Y	11	11 (A->T)	13	20 (T->C)	36 (2A->2G)	17	13 (G->T)	11	14 (A->G)	20	8	11	18
African American	OT05890	X	7	10 (A->T)	11	12	30	16 (G->A)	11	9	16 (A->G)	19	7	6	16
		Y	8	12 (A->T)	12	18	32.2	17	12	10	---	24	8	11	18
African American	OT05897	X	12	11	11	15	27	16	12	8	13 (A->G)	22	7	11	16
		Y	13	12	13	17	30 (A->G)	18	---	---	---	23	8	---	18
African American	OT05898	X	8	12	11	17	29	14 (G->A)	8 (G->T)	8	13 (A->G)	19	8	6	15
		Y	11	13	---	---	32.2	15 (2G->2A)	12	13	15 (A->G)	22	9	9	17
African American	OT05899	X	7	12	9	15	28	13 (2G->2A)	8 (G->T)	11	13	22	7	7	16 (G->A)
		Y	11	---	11	16	32.2	16 (2G->2A)	11	12	14 (A->G)	---	---	10	17
African American	PT84223	X	12	11 (A->T)	11	15	29	17	11	8	14 (A->G)	21	7	8	16
		Y	---	---	13	19	29 (A->G)	17 (2G->2A)	12	10	15 (A->G)	22	---	11	19 (2A->2G)
African American	PT84224	X	10	11 (A->T)	8	16	30 (A->G)	15	12	10	14 (A->G)	22	7	11	15
		Y	12	12	9	17	31	16 (2G->2A)	---	---	---	26	---	---	17
African American	PT84225	X	10	11 (A->T)	9	15	31	17 (G->A)	12	10	13 (A->G)	24	7	10	14 (A->G + 2T->2C)
		Y	12	---	12	17	---	18 (G->A)	---	11	---	25	---	12	16 (G->A)
African American	PT84232	X	9	12 (A->T)	11	14	27	15 (2G->2A)	9 (G->T)	8	13 (A->G)	20	7	8	15
		Y	10	13	12	20 (T->C)	28	16 (2G->2A)	10	10	14 (A->G)	25	---	9	19
African American	PT84234	X	10	13	9	15	29	15 (2G->2A)	12	10	13	19.2	6	6	16
		Y	12	---	11	16	31	16	13	11	15 (A->G)	25	7	---	16 (G->A)
African American	PT84236	X	7	12	11	12	28	16 (2G->2A)	12 (G->T)	8	12 (A->G)	25	7	9	14 (T->C)
		Y	12	13	---	23	29	16 (G->A)	13	9	14 (A->G)	26	---	---	17
Caucasian	MT94859	X	10	9	9	13	29	16	11	9	11	20	6	8	18
		Y	13	11	12	17	30	17 (G->A)	13 (G->T)	11 (T->A)	---	26	9	11	---
Caucasian	UT57300	X	10	8	9 (A->G)	10	28	15	12	8	10	20	9.3	8	15 (G->A)
		Y	12	12	12	13	---	16 (G->A)	---	13	15 (A->G)	21	---	9	19
Caucasian	UT57301	X	11	8	11	15	28	15 (G->A)	11 (G->T)	8	13 (A->G)	23	6	8	17
		Y	12	---	12	17	---	16 (G->A)	13	11	14 (A->G)	25	---	---	18
Caucasian	UT57302	X	10	11 (A->T)	11	14	28	15 (G->A)	11	8	10	21	8	8	14 (G->A+T->C)
		Y	12	12 (A->T)	---	17	29	17	---	13	13 (A->G)	24	9	9	17
Caucasian	UT57318	X	11	11 (A->T)	11	16	25.2	15 (G->A)	10	10	12	21	9.3	8	15 (G->A)
		Y	13	12 (A->T)	---	18	30	16 (G->A)	12 (G->T)	11	---	22	---	11	17
Caucasian	WA29594	X	11	11	11	12	30	17	11	7	12	22	6	8	14 (A->G + 2T->2C)
		Y	12	12	---	15	30.2 (G->A)	18	12	11	13 (A->G)	25	9	---	14 (G->A+T->C)
Caucasian	WA29612	X	11	11 (A->T)	11	12	28	14 (G->A)	12	11	13 (A->G)	22	6	8	14 (G->A+T->C)
		Y	12	13	12	14	30 (A->G)	17 (G->A)	---	12	---	23.2	9.3	11	19
Caucasian	WT51342	X	10	11	12	13	29	18	12	9	12	24	9	9	18
		Y	12	14	13	14	31 (G->A)	---	13	12	14 (A->G)	25	9.3	11	---
Caucasian	WT51343	X	11	11	11	14	28	16 (G->A)	11	10	12	22	6	8	17
		Y	12	13	---	16	31.2	17 (G->A)	12	12 (T->A)	13	---	7	---	18
Caucasian	WT51345	X	11	11 (A->T)	10	13	29	15 (G->A)	11	8	13 (A->G)	20	7	8	17
		Y	12	---	13	---	---	16 (G->A)	---	10	14	22	9.3	---	19
Caucasian	WT51362	X	11	8	9	16	30	16	11	10 (T->A)	10	23	9.3	10	17
		Y	12	13	11	19	31	17 (G->A)	12	12 (T->A)	---	24	---	11	18
Caucasian	WT51373	X	10	11 (A->T)	11	14	28	15 (G->A)	10	8	11	21	8	8	15 (G->A)
		Y	12	12 (A->T)	13	17	---	18	11	11	14 (A->G)	22	9	11	17 (G->A)
Caucasian	WT51378	X	10	8	9	12	30	15	11	9	13 (A->G)	19	6	8	16
		Y	---	12	11	15 (T->G)	31 (G->A)	18	---	---	---	23	9	---	16 (G->A)
Caucasian	WT51381	X	12	8	9	12	30 (A->G)	15 (G->A)	12	10	10	22	6	8	15 (G->A)
		Y	---	11	11	18	---	17	14	---	16 (A->G)	24	---	9	18
Caucasian	ZT81387	X	11	9	12	16	28	18	11	10 (T->A)	13 (A->G)	21	7	6	16
		Y	---	13	13	18	32.2	---	11 (G->T)	11	15 (A->G)	22	---	9	19
Hispanic	GT37778	X	10	9	10	14	28	17 (G->A)	11	9	14 (A->G)	23	7	9	15
		Y	12	13	12	20	30 (A->G)	18	---	12	---	24	---	11	17 (G->A)
Hispanic	GT37812	X	12	12	11	15	29	14 (G->A)	14	8	13	22	6	11	15
		Y	13	13	13	---	32.2	15 (2G->2A)	14 (G->T)	12	13 (A->G)	---	9.3	---	16
Hispanic	GT37828	X	10	9	9	14	31.2	15 (G->A)	11	11	10	22	7	8	16
		Y	---	14	11	17	31.2 (G->A)	16 (2G->2A)	12 (G->T)	12	15 (A->G)	23	---	---	18
Hispanic	GT37900	X	11	11	10	12	29	14 (G->A)	11	9	13 (A->G)	23	6	11	17
		Y	12	12	12	13	31.2	17 (A->G)	---	11	14	26	7	---	19
Hispanic	GT37913	X	11	11	11	13	29	16	7	10	12	19	6	8	16 (G->A)
		Y	12	---	---	15	31 (G->A)	17 (G->A)	11	13	13 (A->G)	22	9.3	11	17
Hispanic	JT52076	X	12	8	11	16	30	15 (G->A)	11	11	12	21	8	8	15 (G->A)
		Y	---	---	12	21	32.2	16	---	12	14 (A->G)	22	9.3	11	18
Hispanic	TT51422	X	10	13 (A->T)	13	15	29	16	11	10	11	20	6	8	16
		Y	12	---	---	18	31 (G->A)	17	13	12	14 (A->G)	24	8	11	17 (G->A)
Hispanic	TT51435	X	9	9	11	12	28	15 (G->A)	10	10	10	23	7	8	16
		Y	12	10 (A->T)	14	17	30.2	16 (G->A)	13 (G->C)	11	12	26	9.3	9	17
Hispanic	TT51483	X	11	11	11	18	30 (A->G)	14 (G->A)	12	8	13 (A->G)	21	9	9	14 (A->G + 2T->2C)
		Y	---	11 (A->T)	---	19	31	18	12 (G->T)	10	15 (A->G)	26	9.3	10	18
Hispanic	ZT80786	X	10	9	9	15	29 (G->A)	15 (G->A)	11	9	13 (A->G)	20	7	11	15 (G->A)
		Y	11	12 (A->T)	---	16	30	18	12	11	15 (A->G)	23	8	12	20
Hispanic	ZT80815	X	10	13	10	14	28	14 (G->A)	10	11	13 (A->G)	24	7	10	16 (G->A)
		Y	13	15 (A->T)	12	16	29 (G->A)	15 (G->A)	11	12	---	---	8	11	17
Hispanic	ZT80826	X	10	10	9	12	28	17	9 (G->T)	10	11	21	9.3	8	17
		Y	12	12	11	18	31.2	18 (G->A)	12	12 (T->A)	17 (A->G)	---	---	---	17 (G->A)

Two-fold serial dilution tests were done using two templates (blood samples N31774 and SC35495) from 1 ng to 62.5 pg input per reaction. Full, correct profiles for both templates were produced at 125 pg template per reaction (data not shown). A test set of 50 plates was then produced to evaluate on diverse source samples. The primers used were those in Table 3. However, the layout of the plates (relative to STR loci and sample loading) was still identical to the layout shown in Figure 2. B). Plates were created such that 5 μl of template were to be added to each well prior to thermocycling.

Table 5. STR alleles from locus D13S317 that were sequenced.

Allele	Sample
9 (dropped out)	PT84225
9 (dropped out)	TT51422
9 (did not drop out)	TT51435
9 (did not drop out)	ZT80786
10	ZT80826
10 (A->T)	TT51435
11 (A->T)	PT84225
12	ZT80826
12 (A->T)	ZT80786
13	GT37778
13 (A->T)	TT51422

To test the new primer pair set for performance upon diverse templates, a 40-sample subset of population reference samples obtained from NIST[53] was run and resulting profiles were compared to previously-obtained profiles[48] for concordance (Table 4). For the 40 samples tested, all alleles called were identical to previous calls. However, two instances of allele 9 from locus D13S317 dropped out with the new plates (Table 4). To investigate this problem, a primer pair was designed to D13S317 outside the range of all primer pairs we have evaluated in order to amplify the entire target region for cloning and sequencing. Primer pair D13S317_G09017_22_258 (forward primer 5′-GTATCACAGAAGTCTGGGATGTGGAG-3′, reverse primer 5′-GTTGGTCAAATCTCCTCCTTCAACTTG-3′) was used to amplify locus D13S317 from the two samples containing an allele 9 that dropped out (PT84225 and TT51422) and two samples containing an allele 9 that did not drop out (TT51435 and ZT80786). In addition, alleles from 10 to 13 with and without an A→T SNP were amplified for sequencing to verify the existence and position of the A→T SNP in variant alleles of D13S317 (Table 5). For each PCR reaction performed using the samples in Table 5, PCR products were introduced directly into cloning vector pDrive using the pDrive cloning kit (Qiagen) according to the manufacturer's recommendations. Ligation reactions were used to transform Qiagen EZ Competent cells (Qiagen #1016780). After colony growth, individual colonies were picked, replicated onto another agar plate, and swirled directly into pre-made PCR reaction cocktails containing primer pair 2819 (the D13S317 primer pair that correctly amplified the allele 9 variants that dropped out with primer pair 1216). The mass spectrometry assay was then used to screen colonies for isolated alleles. Colonies containing isolated alleles were grown up and plasmids were purified using the Qiagen QIAprep Mini prep spin kit (Qiagen #27106). Inserts from plasmids were sequenced at Retrogen (San Diego, CA).

Sequenced alleles are shown in Figure 10. Sequence data for allele 9 variants that dropped out clearly suggests that a mismatch on the 3′ end of the reverse primer of primer pair 1216 inhibited amplification. In addition, sequence data suggest that the A→T SNP found in variants of alleles 10, 11, 12 and 13 appear to be in the same location. The variant allele 9, on the other hand, does not look like a simple mutation from the canonical allele 9. The pathway that the allele took to get to its present state is unclear, but it appears closer to an allele 11 containing a deletion downstream of the repeat region (see Figure 10). Interesting, the two samples that had the allele were

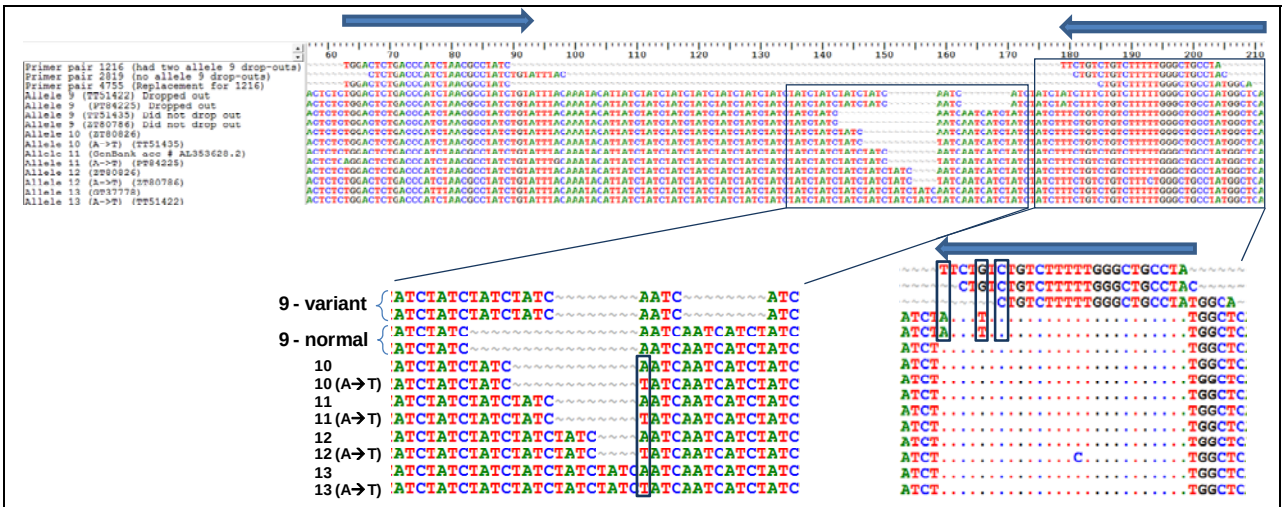


Figure 10. Sequencing of alleles of locus D13S317. Two representatives of allele 9 that dropped out with the new STR primer pairs were sequenced alongside two representative allele 9's that did not drop out. In addition, representatives of alleles 10, 12 and 13 with and without an A→T SNP, and allele 11 with an A→T SNP, were sequenced. The reference sequence GenBank accession #AL353628.2 is an allele 11 and was used for comparison to the sequenced alleles. An additional primer was designed to replace the reverse primer of primer pair 126 that will be tested for incorporation into the STR assay.

Table 6. Primer pair replacements for current STR assay panel

Locus	Previous primer pair	Previous concentration (nM)	Previous triplex	New primer pair	New concentration (nM)	Current triplex	New forward sequence	New reverse sequence
CSF1PO	3882	350	2	4863	250	1	TTGGCATGAAGATATTAACAGTAACTGCCTTC	TCTGTGTCAGACCCTGTTCTAAGTACTTC
D13S317	1216	250	1	4755	400	2	TGGACTCTGACCCATCTAACGCCTATC	TGCCATAGGCAGCCCAAAAAGACAG
D7S820	3885	280	4	4864	280	4	TTGGGAACACTTGTCATAGTTTAGAACGAAC	TGGCCCCTAAATGTTTACTATAGACTATTTAGTGAG
D5S818	4450	460 / 500	4 & 5	4866	360 / 460	4 & 5	TGGGTGATTTTCCTCTTTGGTATCCTTATGTAAT	TCCAATCATAGCCACAGTTTACAACATTTG

from two different population groups (Caucasian and Hispanic), and had an identical sequence throughout the region, suggesting that the variant may be an established allele that has been passed through the human population. The reverse primer of the primer pair for locus D13S317 was modified by moving the 3′ end of the primer back beyond the second mismatched base (see Figure 10). The new reverse primer sequence is 5′- TGCCATAGGCAGCCCAAAAAGACAG-3′.

Primer pairs that were performing poorly or inconsistently relative to other pairs in the triplexes were re-examined a further time for redesign. Primer pairs for CSF1PO, D7S820 and D5S818 were redesigned in addition to the primer redesign for D13S317 that was forced by the rare end mismatch to the reverse primer of primer pair 1216 discovered in two examples of a variant allele 9 (see Figure 10). Redesigned primer pairs are shown in Table 6. After reformulating the primer pair mixes and substituting the D13S317 replacement to fix the problem with the allele 9 variants, the panel of 40 samples from NIST (see Table 4) were retested to verify concordance with the reformulated plate layout. All genotypes were concordant with previous results with respect to base allele calls (not shown). However, the new primer pair for D3S317 produces an allele 9 with a G→T SNP in samples PT84225 and TT51422, which is also predicted from the allelic sequences generated for these samples (Figure 10).

Assay optimization

In an effort to optimize the Ibis STR assay for commercialization, a number of parameters were evaluated (Table 7). For most of these commonly-evaluated factors there are published examples of favorable effects on the PCR, as indicated.

Table 7. Parameters for optimization of the STR assay. Parameters and their potential mechanisms of action/effects on PCR are listed, with citations for selected variables reported to have a positive effect.

Thermocycling Parameters	annealing temperature, time	formation of the primer•target duplex
	ramp rate, melt → anneal	preserve the primer•target duplex
	ramp rate, anneal → extend	maintain the Taq• primer•target complex
	extension time, temperature [54]	optimize primer extension
	number of cycles [54 55]	maximize product levels
Reaction Formulation	$[Mg^{+2}]$ [56, 57 54]	cofactor, divalent counterion
	$[K^+]$, $[NH4^+]$ [56 54]	monovalent counterions
	betaine [58 59 60]	thermostabilizer, cryostabilizer, base pairing effects
	sorbitol	stabilizes polymerase
	Triton X-100	stabilizes polymerase
	BSA [54]	stabilizes polymerase, binds inhibitors
	DMSO (1) [59 60]	perturbs base pairing
	formamide [61]	perturbs base pairing
	[buffer] [54]	empirical
	Taq: vendor, amount [54 55]	product level, specificity, +A levels, cryostability
	[dNTP] [56 48]	product level and specificity
	primer quality	empirical: optimal product levels and baseline
	primer concentration [54]	influences product level and specificity

Optimization focused primarily on product yield (deconvolved peak heights) for each primer pair and inter-locus balance was monitored in triplexed reactions.

Optimizations were performed with donor DNA SC35495 (0.5ng per reaction). This donor is heterozygous for all 14 loci within the assay, providing a maximally diverse target for analysis (Table 8). Evaluations of thermocycling parameters were done in 40ul reaction volumes with 20 mM Tris buffer, 75 mM KCl, 400 mM betaine, 1.5 mM $MgCl_2$, 200 uM each dNTP, 5 units per reaction AmpliTaq Gold, and the primer pair concentrations listed in Figure 11. Evaluations of PCR buffer formulations were done with the primer pair concentrations listed in Figure 11.

Table 8. Genotype of donor DNA SC35495.

locus	allele 1	allele 2
AMEL	X	Y
CSF1PO	11	12
D3S1358	17(G->A)	19
D5S818	11	12
D7S820	8	9
D8S1179	12	15 (A->G)
D13S317	11 (A->T)	11
D162539	8	9
D18S51	15	18
D21S11	28	30 (A->G)
FGA	19	23
THO1	6	9.3
TPOX	10	11
vWA	17	18

Although we have noted that immolase (BioLine) used at 1-1.5 U/reaction produces results comparable to AmpliTaq Gold (Applied Biosystems) used at 3-5 U/reaction (and would thus result in cost savings for manufacturing), we have found that pre-fabricated plates containing immolase as part of the reaction mixture stored at -20 °C for several weeks do not perform well when re-thawed for use in our assay. Examination of data produced with kit plates stored frozen with immolase in them suggests that the enzymatic inhibition that requires activation at elevated temperatures (and therefore provides the "hot start" element) is damaged by freezing in reaction buffer. This speculation is not proven conclusively, but is based upon the observation that kit plates containing immolase taken from the freezer produce prohibitively strong dimer and artifact products that substantially interfere with analysis (data not shown), much like using a Taq polymerase without a hot-start modification.

Triplex 1		
pp	locus	conc
3894	vWA	270
4863	CSF1PO	250
3883	D3S1358	380

Triplex 2		
pp	locus	conc
3892	THO1	215
1210	D16S539	285
4755	D13S317	400

Triplex 3		
pp	locus	conc
3893	TPOX	150
3895	AMEL	260
3886	D8S1179	490

Triplex 4		
pp	locus	conc
3883	D3S1358	260
4864	D7S820	280
4866	D5S818	360

Triplex 5		
pp	locus	conc
1210	D16S539	160
3894	vWA	280
4866	D5S818	460

Single-plex 1		
pp	locus	conc
4451	D21S11	250

Single-plex 2		
pp	locus	conc
4448	FGA	250

Single-plex 3		
pp	locus	conc
1205	D18S51	250

Figure 11. STR assay layout going into reaction and thermocyclinig optimization. Primer pair groupings and primer pair concentrations are listed. All primers were designed to minimize non-templated adenylation and concentrations have been optimized for inter-locus balance. Colored cells indicate primer pairs that are redundant between reactions.

Optimization of thermocycling parameters

Early development of the Ibis STR assay involved the use of an MJ Research thermocycler. Subsequent development has focused upon use of an Eppendorf Mastercycler *epGradient S*, which has much faster ramping rates than an MJ thermocycler (maximum ramp rates of 6°C/sec for heating and 4.5°C/sec for cooling). Transitioning of the assay to the Eppendorf thermocycler during development involved slowing down ramping during the denaturation to annealing step to allow primers time to bind their proper targets. The initial STR thermocycling program consisted of [96°$_{10\ min}$ [96°$_{25\ sec}$, 56°$_{1.5\ min}$, 72°$_{30\ sec}$]$_{40}$ 72°$_{4min}$], with a 5% ramp during the melt-anneal

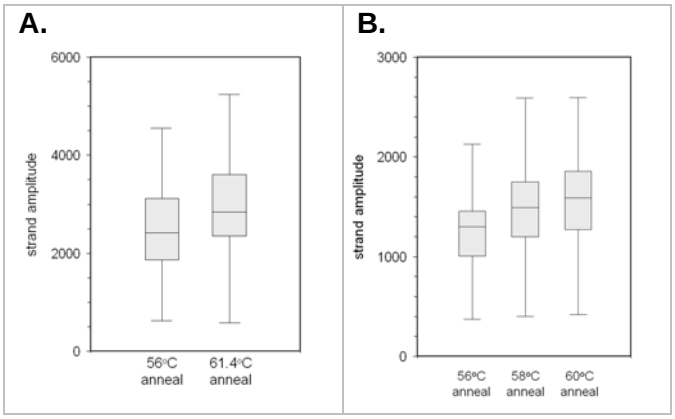


Figure 12. PCR strand amplitudes of products amplified with different annealing temperatures. DNA SC35495 was amplified with various annealing temperatures and the abundance of the PCR products was determined. A.) DNA was amplified in a series of thermocycling programs using a gradient of annealing temperatures. Strand amplitudes are shown for products generated with annealing at 56°C and 61.4°C. B.) Strand amplitudes are shown for SC35495 amplified with either of 3 discrete annealing temperatures: 56, 58, or 60°C.

transition. The thermocycling parameters listed in Table 7 were changed in this basic program in an effort to enhance PCR product levels.

The annealing temperature was evaluated in a 12-point gradient from 53.9 – 65°C. Amplitudes of the PCR products of most alleles were optimal with annealing at 61.4, 62.7, and 63.8°C in the gradient, but with the 2 higher temperatures amplitudes of the FGA and D7 alleles dropped dramatically. Amplitudes of the PCR products produced with annealing at 56°C and 61.4°C are shown in Figure 12A. Overall there was a 20% increase in abundance with the elevated annealing temperature when the ratio of the individual PCR products were calculated, with strands from 2 of the 28 allele products showing slight reductions relative to products from the 56°C anneal. By way of confirmation DNA was amplified with programs incorporating either of 3 discrete annealing temperatures rather than the gradient (56, 58, or 60°C), and comparable results were seen (Figure 12B). These results indicate that incremental gains in amplitude were achieved through fine-tuning the annealing temperature.

Ramp rates in the transitions from melt to anneal and from anneal to extend also can effect PCR performance, presumably by influencing the stability of the primer•target and primer•target•Taq complexes. However, the fastest possible ramps are desirable to minimize the length of the program. The Ibis STR basic thermocycling program was modified with the ramps shown in Figure 13A. These rates are based in part on validated thermocycling protocols in use at Ibis for a variety of different PCR applications. SC35495 DNA was amplified, and levels of all PCR products in the assay responded uniformly to the different amplification schemes, with programs 2, 4 and 5 resulting in higher levels of product (Figure 13B). Results indicated that the assay is tolerant of the standard ramp rate of 4.5°C/sec in the melt-anneal transition, but benefits from a reduced rate of heating in the anneal-to-extend transition. Furthermore the comparison of programs 3 and 4 indicates that a 5% ramp is superior to a 26% ramp in this phase of the program. The ramp settings in program 4 reduce the length of the program by 1 hour, shortening the assay or providing additional opportunity for time-dependent modifications in the thermocycling parameters.

A.

	melt-anneal	anneal-extend
program 1	21%	26%
program 2	21%	5%
program 3	100%	26%
program 4	100%	5%
program 5	5%	5%
program 6	100%	100%

B.

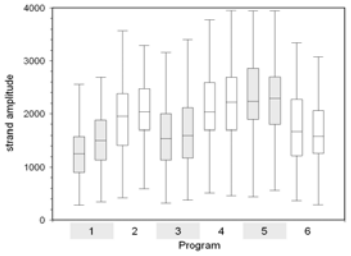


Figure 13. Effect of ramp rates on product levels in the STR assay. A.) The per cent reduction of the 4.5°C/sec cooling rate and the 6°C/sec heating rate of the Eppendorf Master-cycler block is shown for six programs used to evaluate ramp rates for the melt-anneal and anneal-extend transitions. All other thermocycling parameters were as in the basic program. B.) SC35495 DNA was genotyped in duplicate with programs 1-6, and strand amplitudes are plotted for each condition.

Optimization of the reaction formulation

Table 9 lists the PCR formulations used in an evaluation of core buffer components and supplemental additives. The selection of reagents for the optimization of the PCR formulation was guided by our experiences in the development of other Ibis

Table 9. Evaluation of the PCR formulation. DNA SC35495 was genotyped in each of the 43 tabulated PCR formulations. Reagent concentrations are the final concentrations in the reaction.

Mix	Tris mM	$MgCl_2$ mM	Betaine M	KCl mM	Sorbitol mM	NH_4Cl mM	BSA ug/ul	Tx100 %	DMSO %
1	20	1.5	0.4	50	--	--	--	--	--
2	20	1.5	0.4	50	--	5	--	--	--
3	20	1.5	0.4	50	--	--	0.5	--	--
4	20	1.5	0.4	50	--	--	--	0.1	--
5	20	1.5	0.4	50	--	--	0.5	0.1	--
6	20	1.5	0.4	50	--	--	--	--	1
7	20	1.5	0.4	50	--	--	--	--	5
8	20	1.5	0.4	75	20	--	--	--	--
9	20	1.5	0.4	75	20	5	--	--	--
10	20	1.5	0.4	75	20	--	0.5	--	--
11	20	1.5	0.4	75	20	--	--	0.1	--
12	20	1.5	0.4	75	20	--	0.5	0.1	--
13	20	1.5	0.4	75	20	--	--	--	1
14	20	1.5	0.4	75	20	--	--	--	5
15	14	1.5	0.28	52.5	14	--	--	--	--
16	16	1.5	0.32	60	16	--	--	--	--
17	18	1.5	0.36	67.5	18	--	--	--	--
18	24	1.5	0.4	75	20	--	--	--	--
19	28	1.5	0.4	75	20	--	--	--	--
20	32	1.5	0.4	75	20	--	--	--	--
21	36	1.5	0.4	75	20	--	--	--	--
22	40	1.5	0.4	75	20	--	--	--	--
23	20	1.5	--	--	--	--	--	--	--
24	20	1.5	--	50	--	--	--	--	--
25	20	1.5	--	75	--	--	--	--	--
26	20	1.5	--	50	--	5	--	--	--
27	20	1.5	--	75	--	5	--	--	--
28	20	1.5	--	--	--	--	0.5	--	--
29	20	1.5	--	--	--	--	--	0.1	--
30	20	1.5	--	--	--	--	0.5	0.1	--
31	20	1.5	--	50	--	--	0.5	--	--
32	20	1.5	--	75	--	--	0.5	--	--
33	20	1.5	--	50	--	--	--	0.1	--
34	20	1.5	--	75	--	--	--	0.1	--
35	20	1.5	--	50	--	--	0.5	0.1	--
36	20	1.5	--	75	--	--	0.5	0.1	--
37	20	1.5	--	--	--	--	--	--	5
38	20	1.5	--	50	--	--	--	--	5
39	20	1.5	--	75	--	--	--	--	5
40	20	1.5	--	--	--	--	0.5	--	5
41	20	1.5	--	--	--	--	--	0.1	5
42	20	1.5	--	50	--	--	0.5	--	5
43	20	1.5	--	50	--	--	--	0.1	5

assays, the specific requirements of the Ibis STR assay, and published reports such as those noted in Table 7. Management of non-templated nucleotide addition by Taq is a key feature of the Ibis STR assay formulation. This is achieved at the level of primer design using a motif that is biased against non-templated 'A' additions, and also by using a relatively low concentration of 1.5 mM Mg^{+2}. DNA SC35495 was amplified in each of the 43 formulations listed in Table 9. Mix 8 is the base Ibis base formulation that and served as a benchmark for the other mixes. Results did not indicate an obvious reproducible advantage to additional additives beyond the basic Ibis formulation (data not shown) and reaction formulation #8 from Table 9 was moved forward for commercialization.

Final optimization of STR reactions for kit transition to manufacturing

The primary phase of assay development utilized DNA template SC35495, which is heterozygous at all 14 loci targeted by the assay. For final refinement of the assay going into commercialization, a panel of 53 highly-purified human DNA samples was prepared from blood as outlined in Figure 14. This DNA has served as benchmarking material for testing STR assay conditions in the final phase of assay development and validation. During the course of testing the assay on multiple templates, concordance studies, pilot manufacturing and stability studies, a small number of modifications were

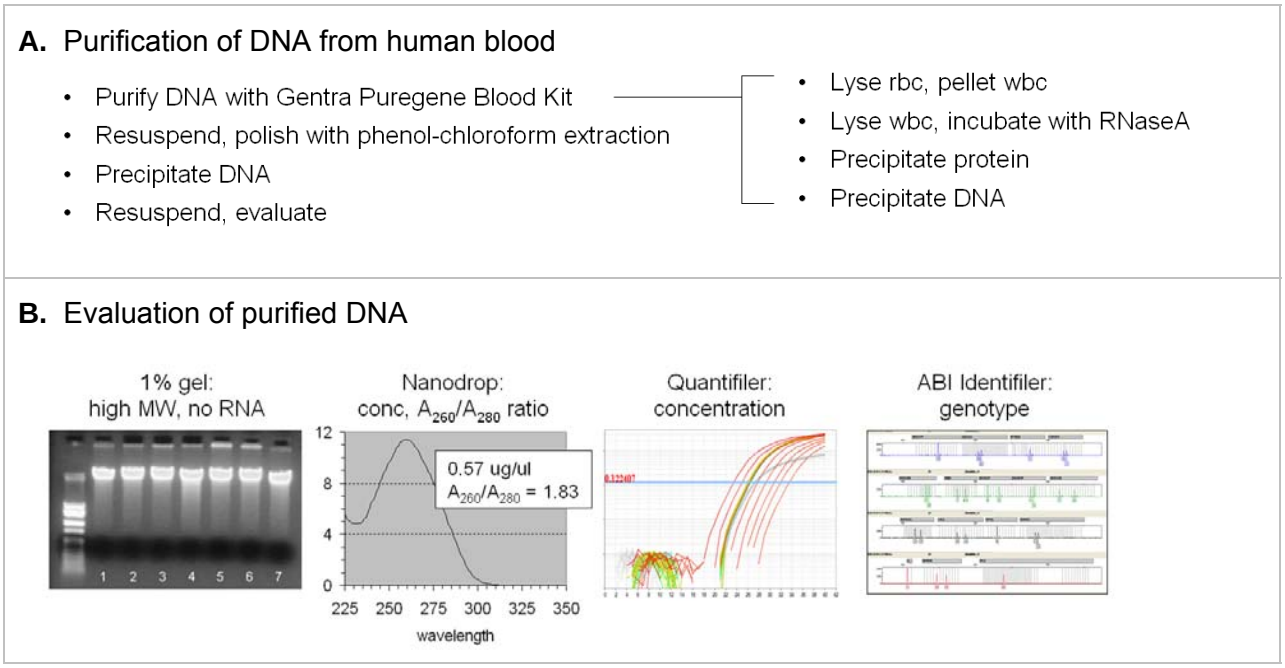


Figure 14. Isolation and characterization of high-quality DNA from human blood. A.) DNA was purified from 53 human blood samples with the method shown. B.) Purified DNA was characterized as illustrated. Gel electrophoresis was used to determine if the DNA was fragmented or contaminated with RNA (which would be evident at the migratory front). Absorbance was used to quantify the nucleic acid and to obtain a measure of its purity with the A_{260}/A_{280} ratio. Typically samples gave ratios of 1.8. The ABI Quantifiler assay gave a second measure of concentration commonly used by the forensic community. The ABI Identifiler assay was used to obtain the sample genotype, providing a benchmark genotype for comparison with results from the Ibis assay.

made to the assay layout and thermocycling parameters. The primer pair for FGA was modified to increase overall product output, and the primer pair for D21S11 was modified to reduce observations of stutter products (not shown). During stability studies performed concomitant to the first formal kit run in the Ibis manufacturing facility, reaction performance with the modified D21S11 became evident after storage for 12 weeks at -20 °C, forcing a reversion of the D21S11 primer pair to a previous version for which acceptable performance was noted after nearly a year storage at -20 °C (not shown). Final primer pairs and final concentrations going into manufacturing are shown in Figure 15.

A.

Triplex 1		
PP	locus	conc
4863	CSF1PO	305
3883	D3S1358	305
3894	vWA	290

Triplex 5		
PP	locus	conc
1210	D16S539	140
4866	D5S818	560
3894	vWA	200

Triplex 2		
PP	locus	conc
4755	D13S317	530
1210	D16S539	220
3892	THO1	150

Singleplex 1		
PP	locus	conc
4451	D21S11	300

Triplex 3		
PP	locus	conc
3895	AMEL	200
3886	D8S1179	600
3893	TPOX	100

Singleplex 2		
PP	locus	conc
4976	FGA	300

Triplex 4		
PP	locus	conc
3883	D3S1358	145
4866	D5S818	395
4864	D7S820	360

Singleplex 3		
PP	locus	conc
1205	D18S51	300

B.

Primer pair number	Locus	Primer Sequences
4863	CSF1PO	TTGGCATGAAGATATTAACAGTAACTGCCTTC TCTGTGTCAGACCCTGTTCTAAGTACTTC
3883	D3S	TTGAAATCAACAGAGGCTTGCATGTAT TTGACAGAGCAAGACCCTGTCTCAT
3894	vWA	TTGGGGAGAATAATCAGTATGTGACTTGGATTG TTGGGTGATAAATACATAGGATGGATGGATAGATGG
4755	D13S317	TGGACTCTGACCCATCTAACGCCTATC TGCCATAGGCAGCCCAAAAAGACAG
1210	D16S539	TCTTCCTCTTCCCTAGATCAATACAGACAG TACCATCCATCTCTGTTTTGTCTTTCAATG
3892	THO1	TTGGAAATCAAAGGGTATCTGGGCTCTGG TTCGCTGGTCACAGGGAACACAGAC
3895	AMEL	TTGCCCTGGGCTCTGTAAAGAATAGTG TTGCATCAGAGCTTAAACTGGGAAGCTG
3886	D8S1179	TTGGGGTTTTGTATTTCATGTGTACATTCGTATC TTGGGTACCTATCCTGTAGATTATTTTCACTGTGG
3893	TPOX	TTGGCACAGAACAGGCACTTAGGGA TTGGTGTCCTTGTCAGCGTTTATTTGCC
4866	D5S818	TGGGTGATTTTCCTCTTTGGTATCCTTATGTAAT TCCAATCATAGCCACAGTTTACAACATTTG
4864	D7S820	TTGGGAACACTTGTCATAGTTTAGAACGAAC TGGCCCCTAAATGTTTACTATAGACTATTTAGTGAG
4451	D21S11	TTTTCCCAAGTGAATTGCCTTCTATC TTGAGGTAGATAGACTGGATAGATAGACGA
4976	FGA	TCCCCAGGCATATTTACAAGCTAGTTTC TGTGATTTGTCTGTAATTGCCAGCAAAAAAG
1205	D18S51	TGTGGAGATGTCTTACAATAACAGTTGCTACTA TCTGAGTGACAAATTGAGACCTTGTCTC

Figure 15. Assay layout of the finalized 14-locus Ibis STR assay. A.) Primer pair groupings and concentrations are listed. Primers were designed to minimize nontemplated adenylation and their concentrations (in triplexes) have been optimized for interlocus balance. B.) Sequences of primers in the final assay layout.

After initial optimization of thermocycling parameters using the single donor template (SC35495), final optimizations were performed using a set of six human DNA samples giving representation of loci most affected by stutter products (primarily in loci D21S11 , D8S1179, D3S1358, vWA, and D7S820). Thermocycling parameters finalized for use on the Eppendorf MasterCycler *epGradient S* and Eppendorf MasterCycler ProS thermocyclers (labeled as "auSTR_PCR_V01" in our project plan for commercialization) are [$96^{\circ}_{10\ min}$, [$96^{\circ}_{25\ sec}$, $60^{\circ}_{45\ sec}$, $72^{\circ}_{2\ min}$]$_{40\ cycles}$, 72°_{4min}, 96°_{10min}], using a 100% ramp rate for the melt-anneal transition (6°C/sec) and a 5% ramp rate for the anneal-extend transition (0.225°C/sec).

Each of the five multiplexes in the Ibis STR assay targets three loci (Figure 15). Within a balanced multiplexed reaction the signals from the three component loci should be similar, with each locus contributing 33% of the total signal in the reaction. Under a given set of reaction and thermocycling conditions primary mutable determinant of interlocus balance in the multiplex reactions is the relative concentration of each of the

primer pairs. Optimizations of the primer concentrations within these reactions were evaluated using interlocus balance as the primary metric. Iterative adjustments in the primer concentrations were made and interlocus balance was evaluated. For this

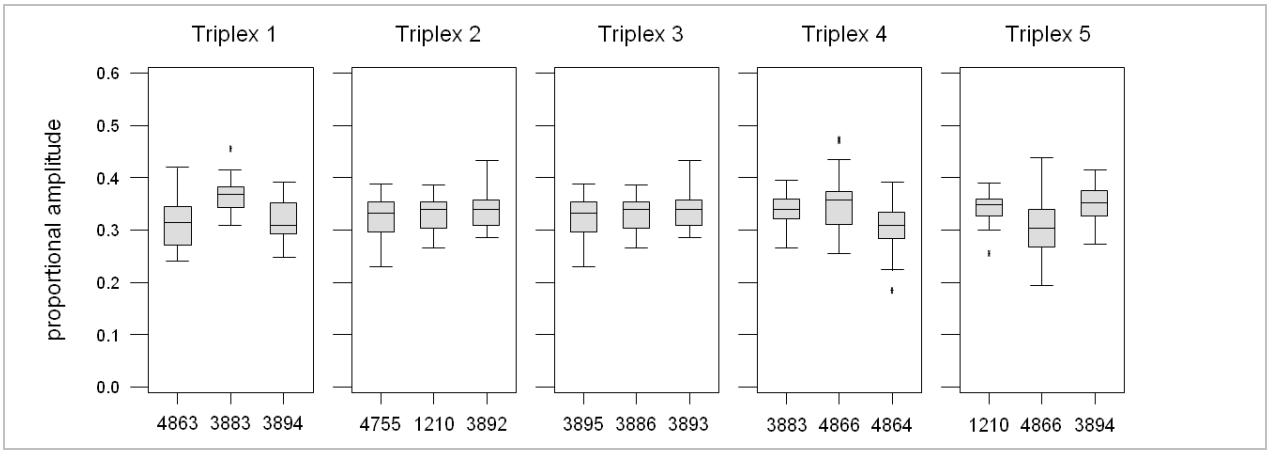


Figure 16. Interlocus balance of the five multiplexed reactions. The five multiplexed assay reactions were formulated with the indicated primer pairs at the concentrations listed in Table 10. A panel of 24 huDNA samples was genotyped and a summary of the interlocus balance is plotted above; median values appear in Table 10.

calculation, strand amplitudes were averaged to give the allele amplitude. The sum of the allele amplitudes was taken as the locus amplitude, and the sum of the locus amplitudes gave the total amplitude of the multiplexed reaction. Interlocus balance then was calculated by dividing the locus amplitudes by the multiplex amplitude, giving three proportions for each multiplex, ideally with each proportion representing 33% of the total. A panel of 24 human DNA samples was analyzed with each of the multiplex formulations, and the interlocus balance was calculated for each donor in each reaction; summaries appear in Figure 16 and Table 10.

Table 10. Proportional amplitudes as a measure of interlocus balance. Multiplexed reactions were formulated as tabulated below, and 24 human DNA samples were analyzed. Median proportional amplitudes derived from the analysis shown in Figure 16 are tabulated (Median Proportion).

Reaction	Locus	PP	Conc (nM)	Median Proportion
Triplex 1	CSF1PO	4863	305	31.5%
	D3S1358	3883	305	36.9%
	vWA	3894	290	30.8%
Triplex 2	D13S317	4755	530	33.2%
	D16S539	1210	220	33.9%
	THO1	3892	150	33.9%
Triplex 3	AMEL	3895	200	35.1%
	D8S1179	3886	600	28.7%
	TPOX	3893	100	35.7%
Triplex 4	D3S1358	3883	145	34.0%
	D5S818	4866	395	35.8%
	D7S820	4864	360	30.9%
Triplex 5	D16S539	1210	140	34.8%
	D5S818	4866	560	30.5%
	vWA	3894	200	35.1%

Applicability of the Ibis STR assay to FTA paper storage

The applicability of Ibis forensic genotyping methods has been evaluated in a number of other projects, and forensic genotypes have been obtained from saliva samples, buccal swab samples, DNA extracted from various archival matrices, and whole genome amplification products. We next evaluated the compatibility of FTA-archived blood samples with the current Ibis STR assay. A set of ten blood samples

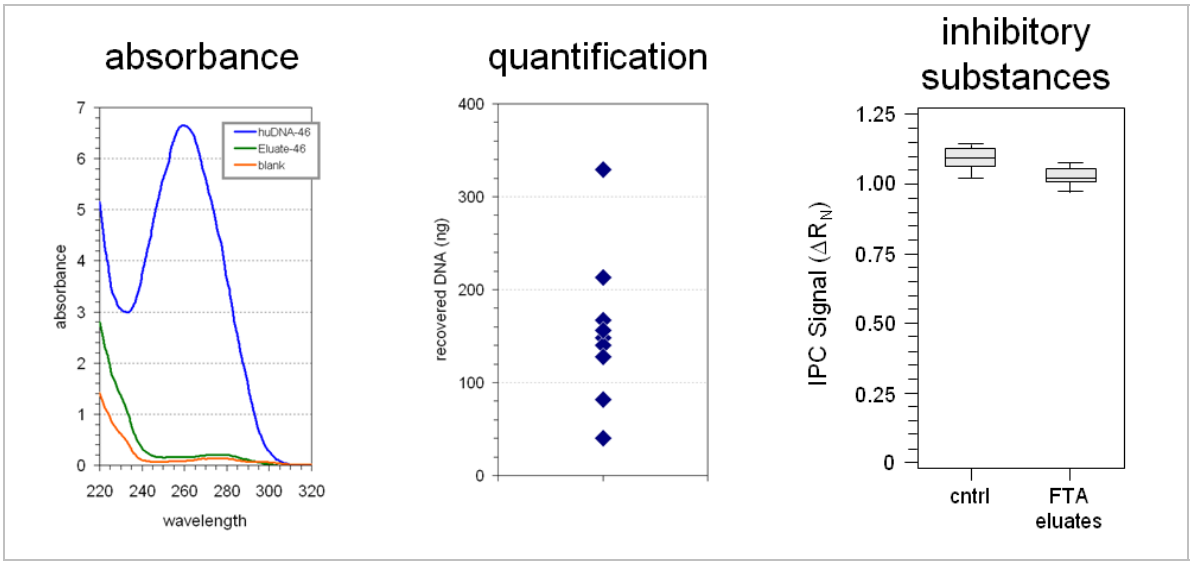


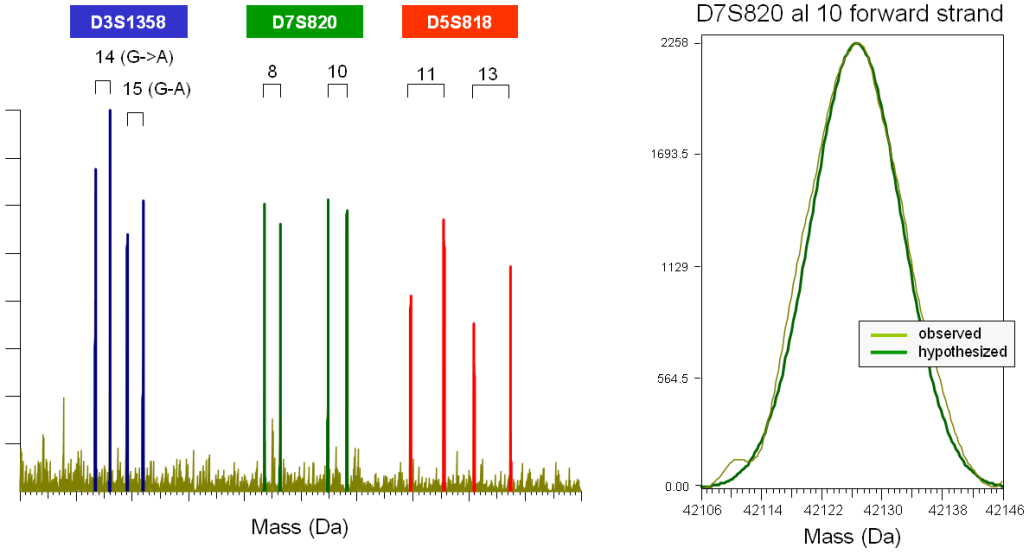
Figure 17. Characterization of FTA eluates. Discs were punched from ten FTA blood spots plus three blank FTA cards, washed, and eluted as described in the text. Absorbance spectra were taken and samples were analyzed with ABI's Quantifiler assay. The left figure shows the spectra of DNA purified from the blood of donor 55-25578 (—), the FTA eluate from the blood spot of donor 55-25578 (—), and the eluate from a blank FTA card (—). There was a high background not only with the eluate of the sample disc, but the blank as well, indicating that the FTA matrix materials contributed to the absorbance measurement. The middle figure shows the total ng eluted from 10 blood-spotted FTA discs as determined with the Quantifiler assay. Recoveries ranged from 40 to 330ng per 3mm disc. On the right are normalized fluorescence readings of the internal positive control from the Quantifiler kit, taken to evaluate whether inhibitory substances were present in the eluates. After quantification in the real-time assay, a post-amplification reading of the endpoint VIC fluorescence was taken; levels of IPC fluorescence in the eluates were 94% of the assay controls, suggestive of a very slight inhibitory effect with the eluates.

was spotted on Whatman FTA Classic Cards in 25 μL volumes and allowed to dry at ambient temperature for one hour before extended storage. Donor DNAs were purified from the remaining blood as outlined in Figure 14, and served as control samples for the subsequent genotype analysis. After twelve days, discs were punched from the FTA blood spots using a Harris Uni-Core 3mm Punch. Discs were washed with FTA Purification Reagent and TE buffer as specified by the manufacturer, and allowed to air dry overnight. Since the disc would interfere with the post-PCR cleanup and spray of the sample on the mass spectrometer, archived DNA was eluted from the FTA disc prior to amplification in the PCR. The manufacturer's protocol was followed for alkaline elution of DNA from the FTA matrix. A 35 μL volume of 0.1N NaOH, 0.3mM EDTA, pH

13.0 was added to the disc. After a 5-minute incubation at ambient temperature, 65 μL of a neutralization solution was added (0.1M Tris-HCl, pH7.0). Samples were vortexed and after ten minutes the disc was removed, and the eluates were ready for analysis.

Eluates were characterized by absorbance and quantified with ABI's Quantifiler assay (Figure 17). As might be expected there was background absorbance associated with the eluates, resulting in a sevenfold overestimation of DNA content compared to the Quantifiler values, on average. There was a slight indication of inhibition associated

A. Deconvolved spectra from a multiplexed reaction genotyping an eluate of FTA-spotted blood from donor 55-25578



B. Deconvolved spectra from a multiplexed reaction genotyping a highly purified DNA sample from donor 55-25578

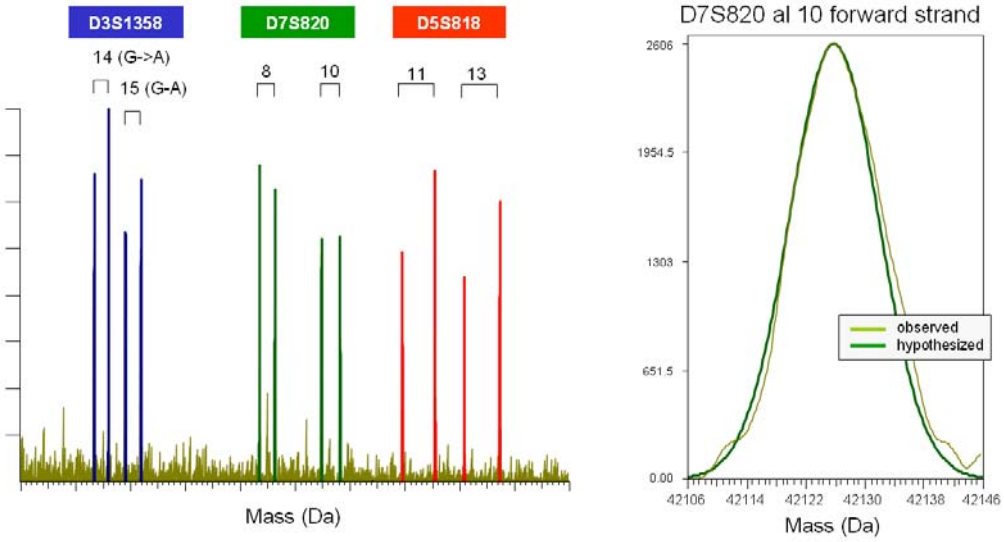


Figure 18. Analysis of FTA-archived blood samples with the Ibis STR assay. FTA blood spots were prepared with ten donor samples. DNA was eluted from FTA card punches as described in the text, and 1ng eluted DNA was added to each of the 8 wells of the Ibis STR assay. Samples were analyzed and deconvolved spectra from Triplex 4 are shown for the FTA eluate (top) and purified DNA (bottom) derived from donor 55-25578. On the right are expanded views of D7S820 allele 10 forward strand, showing the observed and theoretical mass distributions for both samples.

with the eluates as measured by a very modest decrease in the endpoint fluorescence of the internal positive control in the Quantifiler assay. Eluates were genotyped in the Ibis STR assay using 1 ng DNA (as measured by the Quantifiler assay) in each of the 8

wells of the assay. All samples gave full STR profiles which were in complete agreement with the STR profiles obtained from the corresponding DNA purified from the blood sample. A comparison of deconvolved spectra from the Triplex 4 reaction is shown in Figure 18, with results from the FTA eluate *versus* the purified DNA for donor 55-25578 in the top and bottom panels, respectively. Detailed views of the spectra are shown on the right; it can be seen that the observed mass distribution for the forward strand of D7S820 allele 10 is matched very well by the theoretical distribution, not only in the analysis of the purified DNA, but for the FTA eluate as well. These results indicate that the matrix and chemistry associated with FTA sample archival are compatible with the Ibis STR assay.

Assay kit production

Ibis assays are configured in a 96-well plate format and are fabricated in a highly automated process. During the term of this NIJ contract the production of the STR assay was scaled up from benchtop production of 10-20 plates to fabrication of 200 plates in the Ibis Pilot Manufacturing Suite. Production of the STR kit had been transferred to the Ibis Manufacturing group, and three kit production runs have been completed with 200-500 plates produced per run. As of September 2010 the Ibis Manufacturing group projected production of 2,920 plates to meet existing commitments for the year 2011.

Implicit in the transfer of the assay to Manufacturing has been the development of quality control metrics and release specifications for the production and lot testing of kitted product. With commercialization the assay falls under the QA/QC policies and procedures in place at Ibis Biosciences and Abbott Molecular, and documentation of the production and further development of the assay from a QA/QC standpoint also is consistent with the needs of the forensic community.

Assay-specific controls have been integrated into the PCR/ESI-MS analysis stream, including a No Addition Control enabling the monitoring of the prefabricated plates for contaminants, and an ultrapure Negative Control that is packaged using methods that remove the trace contaminants normally detected in association with laboratory plasticware. A panel of purified huDNA samples is used to track assay performance during the kitting process, and the release of a manufactured lot is dependent on analysis specifications of the panel as well.

Developmental validation of the autosomal STR assay

A panel of highly purified DNA samples was prepared for the developmental validation of the STR assay. Human blood was purchased from BioMed Supplies (Carlsbad, CA). DNA was extracted using the Gentra Puregene Blood Kit (Qiagen), and then polished with organic extraction and precipitation. Sample quality was evaluated with gel electrophoresis, and the DNA was quantified by absorbance spectroscopy. Samples were amplified on assay plates kitted in the Ibis Pilot Manufacturing Suite. PCR products were desalted and sprayed on the Ibis T5000 platform. Results were analyzed with the IbisTrack software, and assay outputs were exported to Excel for further analysis. Validation studies of the Ibis STR assay generally followed SWGDAM guidelines for developmental validation. The parameters listed in Table 11 were addressed as described below.

Species specificity

Species specificity was evaluated using a panel of nonhuman DNA: male dog, Zyagen P/N GD-150M; male cat, Zyagen P/N GC-130M; *Escherichia coli* DH5α; *Staphylococcus aureus* USA300; *Aspergillus oryzae*, ATCC P/N 42149D-2; and *Candida albicans*, ATCC P/N MYA-2876D. Each of the non-human DNA samples was analyzed in the Ibis STR assay using 10 ng per reaction in replicates of 6 (4 replicates for PP4451). No detections were evident with any of these samples. Mixtures of the non-human DNA were prepared with human DNA in a 10:1 mass ratio using 10 ng nonhuman DNA with 1 ng human DNA. Full profiles were obtained for the human DNA target, with signal quality equivalent to control samples containing only human DNA, indicating a lack of interference from the nonhuman DNA. Results are summarized in Table 12.

Table 11. Parameters evaluated in the developmental validation of the Ibis STR assay.

Species specificity
Sensitivity studies
Accuracy
Reproducibility
Concordance
Inheritance/population study
Positive and negative controls
Balance
Mixture studies
Assay stability

Table 12. Species specificity of the Ibis STR assay. STR genotypes were determined for 10 ng non-human DNA and for mixtures comprised of 10 ng non-human DNA plus 1ng human DNA.

Sample		Genotype													
huDNA (1ng)	**non-huDNA (10ng)**	**PP3895 AMEL**	**PP4863 CSF1PO**	**PP4755 D13S317**	**PP1210 D16S539**	**PP1205 D18S51**	**PP4451 D21S11**	**PP3883 D3S1358**	**PP4866 D5S818**	**PP4864 D7S820**	**PP3886 D8S1179**	**PP4976 FGA**	**PP3892 THO1**	**PP3893 TPOX**	**PP3894 vWA**
none	Dog	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Cat	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	E. coli	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S. aureus	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	A. oryzae	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	C. albicans	---	---	---	---	---	---	---	---	---	---	---	---	---	---
huDNA-32	Dog	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
	Cat	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
	E. coli	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
	S. aureus	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
	A. oryzae	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
	C. albicans	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18

Sensitivity

Sensitivity was evaluated with an analysis of a dilution series of human DNA samples. A preliminary broad-range dilution series was prepared for six human DNA samples, three of which were heterozygous for all target loci in the assay, and three of which were homozygous for various STR loci. The dilution series ranged from 1 – 500 pg per reaction, increasing in 2-fold increments; an upper level of 50,000 pg (50 ng) DNA per reaction was also analyzed. All samples were analyzed in duplicate. The Ibis STR assay includes redundant reactions for three STR loci (Figure 15), and so there are two opportunities to genotype these particular loci. Therefore results were tallied to capture the total number of allele detections regardless of marker redundancy, and also to capture the aggregate allele calls even if one of the redundant markers showed missing detections ("allele detections" and "allele calls", respectively). Results are summarized in Table 13 and Figure 19. Given the approximate mass of the diploid human genome of 6 pg, generally the results were as expected, with increasing frequencies of dropped detections and missed calls becoming evident with 10 genome copies or less per reaction. Full detections were seen with 125 pg per reaction or more, and partial profiles were evident with all remaining DNA levels, included the lowest input level of 1 pg per reaction. Notably, analysis of 50,000 pg DNA

Table 13. Broad-range sensitivity analysis of the Ibis STR assay.

	Observed/Expected	
pg/well	allele calls	allele detections
50,000	1.0	1.0
500	1.0	1.0
250	1.0	1.0
125	1.0	1.0
62.5	0.989 ± 0.027	0.984 ± 0.022
31.3	0.966 ± 0.029	0.943 ± 0.020
15.6	0.889 ± 0.031	0.859 ± 0.036
7.8	0.711 ± 0.063	0.651 ± 0.063
3.9	0.475 ± 0.060	0.430 ± 0.067
2	0.323 ± 0.049	0.286 ± 0.045
1	0.228 ± 0.047	0.193 ± 0.040

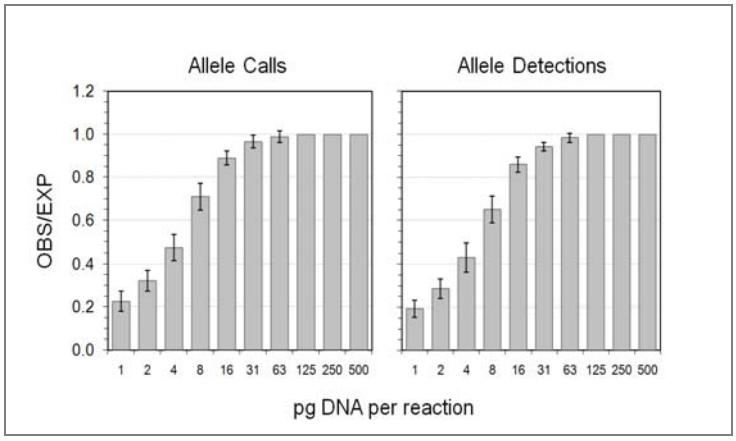


Figure 19. Broad-range sensitivity analysis of the Ibis STR assay. Eight huDNA samples were diluted as described in the text and genotyped in duplicate. The observed number of allele calls was noted for each sample and divided by the expected number of calls, then averaged across replicates and samples. Results are plotted in the left panel. The observed number of allele detections was noted for each sample, divided by the expected number of detections, and averaged across replicates and samples; these values are plotted in the right panel. Note that the results for samples analyzed at 50 ng DNA per well are not included in the plots; all expected calls and detections were made with this level of input DNA.

per reaction gave full detections, with signal quality equivalent to lower input levels. In contrast, DNA inputs greater than approximately 1000 pg per reaction can impact the performance of ABI STR genotyping kits.

A smaller range of input DNA was analyzed to more precisely characterize the sensitivity of the assay. Dilutions of four human DNA samples were prepared at 50, 75, 100, 125, 150, and 250 or 500 pg per reaction. Fifty replicates of each sample were analyzed and the allele calls and allele detections were tallied. For each sample, the observed calls or detections were divided by the expected, and then averaged over the levels of replication of the experiment. Results are summarized in Table 14. Frequencies of detection and calls at all DNA input levels in this range were greater than 0.97. In Table 14 the frequencies of full profile determinations are also shown. In this case the numbers of samples giving a full STR profile were determined at each input level for each DNA sample. These values were divided by the maximal number—50, the number of replicate samples—and averaged across the 4 DNA samples. It can be seen that the frequencies of full profiles decrease more quickly than the frequencies of allele calls, since a single missed allele call out of the 23 – 28 alleles comprising the profile of an individual sample would preclude the determination of a full profile. By this measure the expectation of a full profile with more than 150 pg is 97%, and for 100 pg per reaction it is 93%.

Table 14. Sensitivity analysis of the Ibis STR assay over a narrow range of input DNA.

	Observed/Expected		
pg/well	allele calls	allele detections	full profiles
250/500	0.997 ± 0.014	0.999 ± 0.009	0.979 ± 0.017
150	0.998 ± 0.006	0.994 ± 0.024	0.975 ± 0.030
125	0.996 ± 0.017	0.993 ± 0.024	0.954 ± 0.031
100	0.992 ± 0.031	0.990 ± 0.036	0.934 ± 0.025
75	0.991 ± 0.033	0.981 ± 0.050	0.883 ± 0.029
50	0.983 ± 0.035	0.972 ± 0.047	0.726 ± 0.073

Accuracy

The accuracy of the assay measurements was determined by calculating the measurement error of the mass determinations made for the PCR products. The difference of the expected and observed masses of each strand detection was noted and expressed as a ratio relative to the expected mass, in units of parts per million. Data for this analysis were drawn from the sensitivity study. PCR strand masses ranged from 30,908 – 60,045 Da for the 39,312 detections in this study, with a maximal mass error of 3.6 Da. The average absolute deviation was 11.1 ± 8.9 ppm for all 39,312 detections, similar to the accuracy determined for a mitochondrial typing assay [62]. The distribution of the mass accuracy measurements for the highest input level from each of the replicates of the four human DNA samples is shown in Figure 20, where it is evident that the data appear to be adequately described by the normal distribution, as has been seen with other Ibis assays.

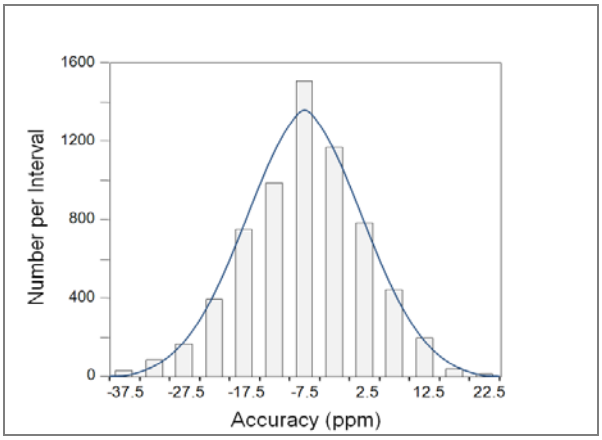


Figure 20. Accuracy of the mass determinations of the Ibis STR assay. Fifty replicates of four huDNA samples were analyzed at 250 or 500 pg per reaction. For each of the 6,576 strand detections the difference of the observed and expected masses was expressed as a ratio relative to the expected mass, in units of parts per million. Histogram intervals increment by the equivalent of 0.5 standard deviation units.

Reproducibility

Reproducibility was determined with data drawn from the highest DNA input levels of the sensitivity study (250 or 500 pg DNA per reaction). There were 6,242 allele detections for the four human DNA samples run in replicates of 50 at this input level. Expected allele detections were 6,248, with 6 missed detections, for detection of 99.9% of all possible alleles for this set of samples (not shown). The instances of the 6 dropout alleles could not be attributed to specific failures in any aspect of the analysis process, i.e., sample application, amplification, post-PCR sample processing, spray on the mass spectrometry platform, or software analysis.

Concordance

Concordance was evaluated by analyzing a panel of 53 human DNA samples in parallel with the Ibis STR assay and with the ABI Identifiler assay. Results generated with the Ibis and AB Identifiler™ assays are shown in Tables 15 and 16, respectively. All samples were analyzed using 1 ng DNA per well. Profiles determined with the Ibis STR assay were made backwards-compatible with Identifiler profiles by using base allele calls, ignoring the SNP-based polymorphisms detected with the Ibis assay. There was 100% concordance of the Ibis STR profiles with the Identifiler assay. As noted in the discussion of primer development above, the D21S11 genotypes listed in Table 23 were obtained with PP4971. This primer pair subsequently was replaced with PP4451. D21S11 genotypes of the 53 human DNA samples were determined with PP4451 during the current reporting period, and results with this primer pair were identical to results obtained with PP4971, and also concordant with the Identifiler genotypes (data not shown).

Table 15. Ibis STR assay genotypes for 53 blood-derived samples prepared at Ibis.

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA
55-24338	X, Y	11, 13	9, 10	10, 11	16, 17	28, 31	14 (G->A), 16 (G->A)	11, ---	8, 9	13 (A->G), 14 (A->G)	19, 20	7, 9.3	9, 11	15, 16
55-24622	X, Y	10, 12	10, 12 (A->T)	11, 12	14, 15	28, 32.2	13, 17	9 (G->T), 11	11, 12	9, 11	20, 25	7, 10	8, 11	14 (A->G + 2T->2C), 15 (G->A)
55-24336	X, Y	10, 11	11, 14	11, 12	17, ---	28, 29	15, 15 (2G->2A)	11, 12	8, 9	12, 15 (A->G)	20, 22	7, 8	8, 10	15 (G->A), 17
55-24413	X, Y	10, 12	11 (A->T), 12 (A->T)	9, 12	14, 16	29 (C->T), 31.2	14 (G->A), 16 (G->A)	11, 12	8 (C->T), 10 (T->A)	11, 13 (A->G)	19, 22	6, 9.3	8, 9	16, 18
55-24187	X, ---	10, 13	11 (A->T), ---	8, 11	14, 15	28, 31 (G->A)	14 (G->A), 18	11, 13	10, 12	10, 15 (A->G)	23, 25	9, 9.3	10, 11	15, 18
55-SMPL6	X, ---	12, 13	12 (A->T), 13	11, 12	18, 21	29, 32.2	15 (G->A), 17 (G->A)	12 (G->T), 14 (G->T)	9, 10	12, 15 (A->G)	20, 21	9.3, ---	8, ---	14 (A->G + 2T->2C), 16
55-24133	X, Y	9, 11	12 (A->T), 14	11, 12	15, 18	28, 30	14 (G->A), 16	12, 13	9, 10	14 (A->G), ---	19, 20	9.3, ---	8, ---	15 (G->A), 17 (G->A)
55-24701	X, ---	10, 12	9, 12	9, ---	14, 15	29, 32.2	16 (G->A), ---	11, 12	10, ---	15 (A->G), ---	24, 25 (C->A)	8, 9.3	8, ---	14 (A->G + 2T->2C), 16
55-24705	X, ---	11, ---	11, 12	10, 12	14, 15	27 (A->G), 31	12 (2G->2A), 17 (2G->2A)	10, 13	8, 9	13 (A->G), 14 (A->G)	22, 23	8, ---	8, 9	14 (A->G + 2T->2C), 16
55-24781	X, ---	10, 11	8, 12 (A->T)	11, 12	12, 13	29, 30	17, 18	10, 13	11, ---	14 (A->G), ---	23, ---	7, 9.3	8, ---	16, 19 (A->G)
55-SMPL11	X, Y	11, 12	11 (A->T), 13	11, 13	13, 19	30, 31.2	15 (G->A), ---	12, 13	11, ---	11, 14 (A->G)	21, 24	6, ---	8, ---	17, ---
SC35495	X, Y	11, 12	11 (A->T), 11	8, 9	15, 18	28, 30 (A->G)	17 (G->A), 19	11, 12	8, 9	12, 15 (A->G)	19, 23	6, 9.3	10, 11	17, 18
SC48046	X, Y	10, 11	8, 13 (A->T)	11, ---	12, 18	28, 30	15 (G->A), 17	12, 13	10, ---	13 (A->G), 15 (A->G)	23, 24	7, 9.3	8, 11	17, 19
072109B	X, Y	10, 11	12 (A->T), 12	10, 11	12, 17	31, 33.2	16 (G->A), 17 (G->A)	12 (G->T), 12	8, 10	13, 15	21, 25	6, 7	8, 11	14 (A->G + 2T->2C), 17
55-24867	X, Y	10, 12	9, 11	12, 13	16, ---	29, 32 (A->G)	15 (G->A), 17 (G->A)	12 (G->T), 13	11, ---	13, 15 (A->G)	23, 24	6, 7	8, 11	16, 17
55-24907	X, Y	10, 12	9, 12 (A->T)	11, 13	14, 17	30 (2A->2G), 32.2	15, 17 (G->A)	11, ---	9, 12	12, 14 (A->G)	21, 22	7, 9	8, 11	17, 18
55-24916	X, Y	11, ---	11 (A->T), 11	9, 12	12, 15	30 (A->G), 31.2	15 (G->A), 18 (G->A)	9 (G->T), 11	10, 12	11, 15 (A->G)	21, 24	6, 9.3	8, ---	18, 18 (G->A)
55-25006	X, Y	10, 11	8, 11 (A->T)	8, 11	13, 14	28, 31.2	15 (G->A), 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18
55-25026	X, ---	10, ---	12 (A->T), 13 (A->T)	9, 14	14, 15	30 (A->G), 31.2	16 (G->A), 17 (G->A)	11, ---	9, 12	12, 14 (A->G)	21, 22	7, 9	8, 11	16, 19 (A->G)
55-25108	X, ---	11, 12	11 (A->T), 12	8, 11	12, 13	29, 30	16 (G->A), 16 (2G->2A)	11 (G->T), 11	9, 10 (T->A)	12, 15 (A->G)	20, 24	6, 7	8, 11	15 (G->A), 17
55-25110	X, Y	11, 13	9, 11	11, ---	13, 16	30 (A->G), 30	15 (G->A), 16	12 (G->T), 12	10, ---	12, 13	22, ---	6, 7	8, 11	18, ---
55-25113	X, Y	12, ---	11 (A->T), 12	9, 12	16, 17	29, 30 (A->G)	15 (G->A), 18	11, 12	8, 11 (T->A)	11, 12	20, 22	6, 9	8, 9	17, 19
55-25185	X, Y	10, 11	8, 12	10, 12	12, 13	29, 30 (A->G)	16, 19	11, ---	10, ---	10, 14 (A->G)	24, 25	7, ---	8, 11	18, 19
55-25188	X, Y	11, ---	8, 12 (A->T)	11, ---	14, 16	29, 30 (A->G)	14 (G->A), ---	11, 12	10 (T->A), 12	10, 14 (A->G)	20, 22	6, 7	8, ---	17, ---
55-25192	X, Y	10, 11	11, 12 (A->T)	11, 13	13, 19	31.2, 32.2	15 (G->A), 18	13 (G->T), 13	8, 9	12, 13 (A->G)	23, 25	6, 7	8, ---	16 (G->A), 17
55-25193	X, Y	10, 11	8, 12 (A->T)	9, 11	14, 16	30 (A->G), 30.2 (G->A)	15 (G->A), 17	12 (G->T), 12	12 (T->A), 13	12, 14 (A->G)	20, 22	6, 7	8, ---	18, 19
55-25236	X, Y	10, 12	11, ---	12, ---	13, 15	29, 30	17 (G->A), 18	11, ---	11, 13	13 (A->G), 14	21, 22	9.3, ---	8, ---	14 (A->G + 2T->2C), 16
55-25238	X, Y	10, 11	11, 12 (A->T)	12, 14	12, 22	30 (A->G), 30	16 (G->A), ---	11, 12	9, 10	13 (A->G), 15 (A->G)	19, 23	6, 8	8, ---	16, 18
55-25290	X, ---	11, ---	11, 12	9, 13	13, ---	30, 32.2	18, ---	11, 12	8, 11 (T->A)	14 (A->G), ---	21, 25	8, 9.3	8, ---	17, 20
55-25295	X, ---	11, ---	11, 12	10, 12	14, 16	27 (A->G), 31	12 (2G->2A), 17 (2G->2A)	10, 13	8, 9	13 (A->G), 14 (A->G)	22, 23	8, ---	8, 9	14 (A->G + 2T->2C), 16
55-25307	X, Y	10, 12	11, 11 (A->T)	10, 11	13, 18	33.2, ---	14 (G->A), 16	11 (G->T), 12	7, 8	13, 14 (A->G)	21, 24	6, ---	8, 11	16, 17
55-25356	X, Y	10, 12	8, 10	11, 12	15, 17	27 (A->G), 29	17, 18	10, 13 (G->T)	8, 11	13, 13 (A->G)	22, 25	7, 9	8, 11	15, 18
55-25364	X, Y	10, 12	8, 11 (A->T)	12, ---	13, 16	29, 30	16 (2G->2A), 16 (G->A)	12 (G->T), 13	11, ---	10, ---	24, 28.1	6, 8	8, 11	17, 19
55-25367	X, Y	11, 12	12, 12 (A->T)	12, ---	17, ---	28, 31	15 (G->A), 17 (G->A)	9 (G->T), 10 (G->T)	10, 11	15 (A->G), ---	22, 25	6, 9.3	8, 12	18, 20
55-25378	X, ---	11, 12	10, 11	12, 13	13, 14	28, 30	14 (G->A), 17 (G->A)	10 (G->T), 13	9, 10	9, 13 (A->G)	23, ---	6, ---	8, 10	16, 17
55-25380	X, Y	10, 11	12, 13 (A->T)	9, 12	12, 17	29, ---	15 (G->A), 16 (2G->2A)	12 (G->T), 13	9, 10	14 (A->G), 16 (2A->2G)	22, 25	6, 8	11, ---	16, 18
55-25381	X, Y	11, 12	8, 11 (A->T)	9, 13	14, 17	27 (A->G), 30.2	16, 17	11, 12 (G->T)	10, ---	12, 13	19, 23	8, 9	8, 11	14 (T->C), 18
55-25445	X, Y	11, 14	8, 12	9, 11	12, 15	28, 31.2	15 (G->A), ---	11, 12	7, 9	11, 15 (A->G)	20, 22	9, 9.3	8, ---	17, ---
55-25446	X, ---	10, 12	12 (A->T), 13	11, 12	13, 15	28, 32.2	13, 17	9 (G->T), 11	11, ---	12, 15 (A->G)	22.2, 23	6, 7	10, 11	15, 16
55-25456	X, Y	8, 9	12, ---	10, 13	17, 18	28, 29	15 (2G->2A), 17 (2G->2A)	12, 13	8, ---	13 (A->G), 16 (A->G)	23, 24	6, 8	9, 11	15 (G->A), 16
55-25460	X, Y	11, 13	8, 12 (A->T)	9, 13	14, ---	28, ---	16 (G->A), 17 (G->A)	10, 13	10, 11	11, 13 (A->G)	19, 20	8, 9.3	9, 11	17, 18
55-25461	X, ---	11, 12	12 (A->T), ---	9, 11	12, 14	28, 29	15 (G->A), ---	11, 12 (G->T)	10, ---	14 (A->G), ---	21, 24	6, 7	10, 12	14 (T->C), 15 (G->A)
55-25462	X, ---	11, 13	11, 12 (A->T)	11, ---	12, 13	27 (A->G), 28	15 (G->A), 16 (G->A)	12, 13 (G->T)	9, 13	14 (A->G), 15 (A->G)	21, 24	6, 7	8, 11	15 (G->A), 16
55-25502	X, Y	10, 12	9, 12 (A->T)	11, 13	14, 17	30 (2A->2G), 32.2	15, 17 (G->A)	11, ---	9, 12	12, 14 (A->G)	21, 22	7, 9	8, 11	17, 18
55-25577	X, Y	10, 11	8, 11 (A->T)	8, 11	13, 14	28, 31.2	15 (G->A), 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18
55-25578	X, Y	10, 13	8, 12 (A->T)	11, 13	11, 16	27, 28	14 (G->A), 15 (G->A)	11, 13	8, 10	12 (A->G), 15 (A->G)	19, 21	6, 9	8, 11	18, 19
55-25597	X, Y	10, ---	11, 11 (A->T)	11, 13	12, 13	30 (A->G), 32.2	16 (G->A), 18	11, 13	9, 10	13 (A->G), 14	20, 25	9.3, 10	11, 12	17, 18
55-25600	X, Y	11, ---	11 (A->T), 12 (A->T)	9, 12	14, 15	30, 30 (A->G)	16, 16 (G->A)	8, 13 (G->T)	8, 10 (T->A)	13 (A->G), 16 (A->G)	20, 24	6, ---	8, 11	18, ---
55-25602	X, Y	11, 12	12, 14	12, 13	12, 16	30, 32.2	15 (G->A), 16 (G->A)	10, 11	12, 13 (T->A)	11, 13 (A->G)	22, 24	6, 9.3	8, 11	14 (A->G + 2T->2C), 16
55-25603	X, Y	10, 12	11, 12 (A->T)	9, 11	13, 15	29, 30	15 (G->A), 16 (G->A)	11, 12	10, 11	12, 16 (A->G)	21, ---	9, 9.3	8, 11	16, ---
55-25704	X, ---	11, 12	10, 12 (A->T)	11, 12	10, 16	25 (3A->3G), 29	17, ---	11 (G->T), 12	8, 10 (T->A)	12, 14 (A->G)	20, 21	6, 9	10, ---	16, 17
55-25705	X, Y	10, 11	12, 13	10, 11	16, 19	29, 30.2	16, 17 (G->A)	13, 13 (G->C)	8, 11	13 (A->G), 15 (A->G)	19, 24	7, ---	11, ---	17, 20 (A->G + A->G)
55-25711	X, ---	10, 12	9, 13	12, ---	12, 14	28, 30	15 (2G->2A), 16 (G->A)	11, 12	8, 11	10, 13 (A->G)	21, 25	6, ---	8, 11	16 (G->A), 18

Table 16. Identifiler assay genotypes for 53 blood-derived samples prepared at Ibis.

Sample	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	THO1	TPOX	vWA	D19S433	D2S1338
55-24338	X, Y	11, 13	9, 10	10, 11	16, 17	28, 31	14, 16	11, ---	8, 9	13, 14	19, 20	7, 9.3	9, 11	15, 16	13, 14	17, 19
55-24622	X, Y	10, 12	10, 12	11, 12	14, 15	28, 32.2	13, 17	9, 11	11, 12	9, 11	20, 25	7, 10	8, 11	14, 15	12, 13	17, 18
55-24336	X, Y	10, 11	11, 14	11, 12	17, ---	28, 29	15, ---	11, 12	8, 9	12, 15	20, 22	7, 8	8, 10	15, 17	14.2, ---	17, 19
55-24413	X, Y	10, 12	11, 12	9, 12	14, 16	29, 31.2	14, 16	11, 12	8, 10	11, 13	19, 22	6, 9.3	8, 9	16, 18	14, 15	17, 19
55-24187	X, ---	10, 13	11, ---	8, 11	14, 15	28, 31	14, 18	11, 13	10, 12	10, 15	23, 25	9, 9.3	10, 11	15, 18	14, ---	23, 24
55-SMPL6	X, ---	12, 13	12, 13	11, 12	18, 21	29, 32.2	15, 17	12, 14	9, 10	12, 15	20, 21	9.3, ---	8, ---	14, 16	15, 16.2	19, 24
55-24133	X, Y	9, 11	12, 14	11, 12	15, 18	28, 30	14, 16	12, 13	9, 10	14, ---	19, 20	9.3, ---	8, ---	15, 17	14, 15.2	17, 19
55-24701	X, ---	10, 12	9, 12	9, ---	14, 15	29, 32.2	16, ---	11, 12	10, ---	15, ---	24, 25	8, 9.3	8, ---	14, 16	12, 14	16, 23
55-24705	X, ---	11, ---	11, 12	10, 12	14, 16	27, 31	12, 17	10, 13	8, 9	13, 14	22, 23	8, ---	8, 9	14, 16	15, ---	18, 19
55-24781	X, ---	10, 11	8, 12	11, 12	12, 13	29, 30	17, 18	10, 13	11, ---	14, ---	23, ---	7, 9.3	8, ---	16, 19	13, 15	17, 22
55-SMPL11	X, Y	11, 12	11, 13	11, 13	13, 19	30, 31.2	15, ---	12, 13	11, ---	11, 14	21, 24	6, ---	8, ---	17, ---	14, ---	17, 25
SC35495	X, Y	11, 12	11, ---	8, 9	15, 18	28, 30	17, 19	11, 12	8, 9	12, 15	19, 23	6, 9.3	10, 11	17, 18	12, 15	25, 26
SC48046	X, Y	10, 11	8, 13	11, ---	12, 18	28, 30	15, 17	12, 13	10, ---	13, 15	23, 24	7, 9.3	8, 11	17, 19	13, 14	22, 25
072109B	X, Y	10, 11	12, ---	10, 11	12, 17	31, 33.2	16, 17	12, ---	8, 10	13, 15	21, 25	6, 7	8, ---	14, 17	12, 15	25, ---
55-24867	X, Y	10, 12	9, 11	12, 13	16, ---	29, 32	15, 17	12, 13	11, ---	13, 16	24, ---	9, 9.3	11, ---	16, ---	12.2, 14	23, 25
55-24907	X, Y	10, 12	9, 12	11, 13	14, 17	30, 32.2	15, 17	11, ---	9, 12	12, 14	21, 22	7, 9	8, 11	17, 18	14, ---	17, 19
55-24916	X, Y	11, ---	11, ---	9, 12	12, 15	30, 31.2	15, 18	9, 11	10, 12	11, 15	21, 24	6, 9.3	8, ---	18, ---	12, 13	19, ---
55-25006	X, Y	10, 11	8, 11	8, 11	13, 14	28, 31.2	15, 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18	14, 15.2	19, 25
55-25026	X, ---	10, ---	12, 13	9, 14	14, 15	29, 32.2	16, 19	11, 12	10, 11	14, ---	21, 22	7, 9.3	8, 11	16, 19	14, 15	20, 23
55-25108	X, ---	11, 12	11, 12	8, 11	12, 13	29, 30	16, ---	11, ---	9, 10	12, 15	20, 24	6, 7	8, 11	15, 17	13, 14	23, 25
55-25110	X, Y	11, 13	9, 11	11, ---	13, 16	30, ---	15, 16	12, ---	10, ---	12, 13	22, ---	6, 7	8, 11	18, ---	14, ---	17, 25
55-25113	X, Y	12, ---	11, 12	9, 12	16, 17	29, 30	15, 18	11, 12	8, 11	11, 12	20, 22	6, 9	8, 9	17, 19	12, 16	22, 23
55-25185	X, Y	10, 11	8, 12	10, 12	12, 13	29, 30	16, 19	11, ---	10, ---	10, 14	24, 25	7, ---	8, 11	18, 19	14, ---	17, ---
55-25188	X, Y	11, ---	8, 12	11, ---	14, 16	29, 30	14, ---	11, 12	10, 12	10, 14	20, 22	6, 7	8, ---	17, ---	14, ---	17, 24
55-25192	X, Y	10, 11	11, 12	11, 13	13, 19	31.2, 32.2	15, 18	13, ---	8, 9	12, 13	23, 25	6, 7	8, ---	16, 17	12, 15.2	20, 25
55-25193	X, Y	10, 11	8, 12	9, 11	14, 16	30, 30.2	15, 17	12, ---	12, 13	12, 14	20, 22	6, 7	8, ---	18, 19	13, 14	18, 23
55-25236	X, Y	10, 12	11, ---	12, ---	13, 15	29, 30	17, 18	11, ---	11, 13	13, 14	21, 22	9.3, ---	8, ---	14, 16	14, ---	20, 25
55-25238	X, Y	10, 11	11, 12	12, 14	12, 22	30, ---	16, ---	11, 12	9, 10	13, 15	19, 23	6, 8	8, ---	16, 18	12, 14	17, 21
55-25290	X, Y	11, ---	11, 12	9, 13	13, ---	30, 32.2	18, ---	11, 12	8, 11	14, ---	21, 25	8, 9.3	8, ---	17, 20	15.2, 16	19, 23
55-25295	X, ---	11, ---	11, 12	10, 12	14, 16	27, 31	12, 17	10, 13	8, 9	13, 14	22, 23	8, ---	8, 9	14, 16	15, ---	18, 19
55-25307	X, Y	10, 12	11, ---	10, 11	13, 18	33.2, ---	14, 16	11, 12	7, 8	13, 14	21, 24	6, ---	8, 11	16, 17	12, 15.2	23, 26
55-25356	X, Y	10, 12	8, 10	11, 12	15, 17	27, 29	17, 18	10, 13	8, 11	13, ---	22, 25	7, 9	8, 11	15, 18	14, 15	17, 24
55-25364	X, Y	10, 12	8, 11	12, ---	13, 16	29, 30	16, ---	12, 13	11, ---	10, ---	24, OL*	6, 8	8, 11	17, 19	15.2, ---	16, 18
55-25367	X, Y	11, 12	12, ---	12, ---	17, ---	28, 31	15, 17	9, 10	10, 11	15, ---	22, 25	6, 9.3	8, 12	18, 20	14, 16	20, 23
55-25378	X, ---	11, 12	10, 11	12, 13	13, 14	28, 30	14, 17	10, 13	9, 10	9, 13	23, ---	6, ---	8, 10	16, 17	12, 14	17, 20
55-25380	X, Y	10, 11	12, 13	9, 12	12, 17	29, ---	15, 16	12, 13	9, 10	14, 16	22, 25	6, 8	11, ---	16, 18	13, 13.2	16, 19
55-25381	X, Y	11, 12	8, 11	9, 13	14, 17	27, 30.2	16, 17	11, 12	10, ---	12, 13	19, 23	8, 9	8, 11	14, 18	12, 14	19, ---
55-25445	X, Y	11, 14	8, 12	9, 11	12, 15	28, 31.2	15, ---	11, 12	7, 9	11, 15	20, 22	9, 9.3	8, ---	17, ---	13, 15	24, 25
55-25446	X, ---	10, 12	12, 13	11, 12	13, 14	28, 29	17, 18	12, 13	11, ---	12, 15	22.2, 23	6, 7	10, 11	15, 16	15, 15.2	17, 25
55-25456	X, Y	8, 9	12, ---	10, 13	17, 18	28, 29	15, 17	12, 13	8, ---	13, 16	23, 24	6, 8	9, 11	15, 16	14, 16.2	22, 23
55-25460	X, Y	11, 13	8, 12	9, 13	14, ---	28, ---	16, 17	10, 13	10, 11	11, 13	19, 20	8, 9.3	9, 11	17, 18	14, 16	17, ---
55-25461	X, ---	11, 12	12, ---	9, 11	12, 14	28, 29	15, ---	11, 12	10, ---	14, ---	21, 24	6, 7	10, 12	14, 15	13, 16	20, 26
55-25462	X, ---	11, 13	11, 13	11, 12	14, 17	30, 32.2	15, 17	11, ---	9, 12	12, 14	21, 22	7, 9	8, 11	15, 16	14, 16	21, 23
55-25502	X, Y	10, 12	9, 12	11, 13	14, 17	30, 32.2	15, 17	11, ---	9, 12	12, 14	21, 22	7, 9	8, 11	17, 18	14, ---	17, 19
55-25577	X, Y	10, 11	8, 11	8, 11	13, 14	28, 31.2	15, 16	10, 11	9, 11	13, ---	20, 21	6, 7	8, 11	17, 18	14, 15.2	19, 25
55-25578	X, Y	10, 13	8, 12	11, 13	11, 16	27, 28	14, 15	11, 13	8, 10	12, 15	19, 21	6, 9	8, 11	18, 19	13, 14	20, 24
55-25597	X, Y	10, ---	11	11, 13	12, 13	30, 32.2	16, 18	11, 13	9, 10	13, 14	20, 25	9.3, 10	11, 12	17, 18	13, 15	17, 23
55-25600	X, Y	11, ---	11, 12	9, 12	14, 15	30, ---	16, ---	8, 13	8, 10	13, 16	20, 24	6, ---	8, 11	18, ---	12, 14	17, 23
55-25602	X, Y	11, 12	12, 14	12, 13	12, 16	30, 32.2	15, 16	10, 11	12, 13	11, 13	22, 24	6, 9.3	8, 11	14, 16	14, 16	23, 25
55-25603	X, Y	10, 12	11, 12	9, 11	13, 15	29, 30	15, 16	11, 12	10, 11	12, 16	21, ---	9, 9.3	8, 11	16, ---	12, 13	19, 21
55-25704	X, ---	11, 12	10, 12	11, 12	10, 16	25, 29	17, ---	11, 12	8, 10	12, 14	20, 21	6, 9	10, ---	16, 17	12, 13	19, 20
55-25705	X, Y	10, 11	12, 13	10, 11	16, 19	29, 30.2	16, 17	13, ---	8, 11	13, 15	19, 24	7, ---	11, ---	17, 20	13.2, 14	17, 24
55-25711	X, ---	10, 12	9, 13	12, ---	12, 14	28, 30	15, 16	11, 12	8, 11	10, 13	21, 25	6, ---	8, 11	16, 18	12, 14	24, 25

Inheritance/population studies

The accurate mass determinations made with the Ibis STR assay enable the routine identification of SNPs in the target loci. In the course of the development of the Ibis STR assay the occurrence of STR SNP variants and their inheritance in family sample sets have been examined over multiple sample sets. These studies were done in collaboration with John Planz and Art Eisenberg of the University of North Texas Health Sciences Center (UNTHSC), Fort Worth, TX, John Butler at NIST, and Cecelia Crouse at the Palm Beach County Sherriff's Office (Palm Beach, FL). The data are referenced here in support of the developmental validation of the Ibis STR assay, specifically with regard to SNP detections.

A preliminary determination of the frequency of SNP variants in the CODIS loci was made with a panel of DNA samples derived from 297 Caucasian, 332 African American, and 313 Hispanic individuals. Samples were genotyped with the Ibis STR assay, and SNP polymorphisms were observed in all assay loci except AMEL, THO1, and TPOX. Results appear in Table 34. Note that these samples were run with a different iteration of the assay utilizing slightly different primer pairs, but output results are informatically equivalent to those obtained with the finalized assay.

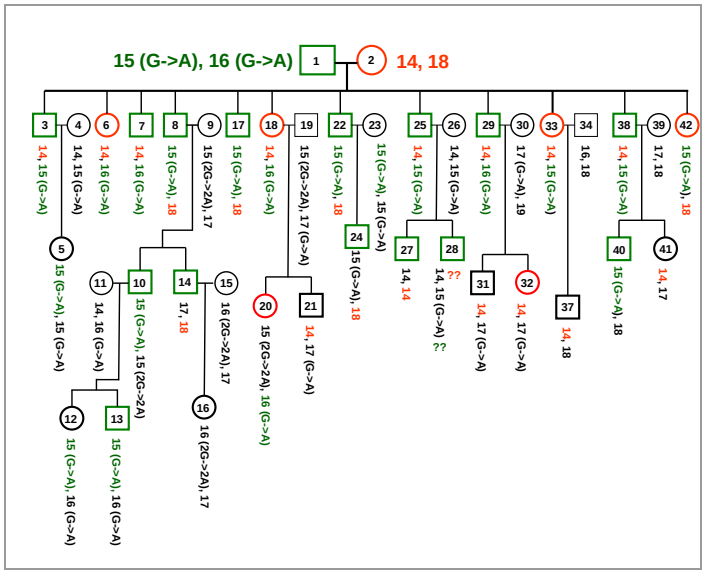


Figure 21. Inheritance of D3S1358 alleles within a 42-sample pedigree set. Genotypes were obtained for each of 42 samples with the Ibis STR assay. Passage of the D3S1358 alleles is illustrated above, with paternal alleles (sample 1) colored green, maternal alleles (sample 2) colored red, and alleles originating from outside of the primary pedigree colored black.

SNP assignments could be informative in situations benefitting from additional discriminatory power, such as where partial profiles are obtained, or with analysis of inheritance. Figure 21 shows an example of the passage of D3S1358 alleles through a family of 42 individuals. SNPs were evident in both D3S1358 alleles of the grandfather (sample 1), and consequently all of his children acquired one of these two SNP variants. Notably, a grandchild (sample 10) acquired allele 15 (G→A) from his grandfather, together with allele 15 (2G→2A) from outside of the primary pedigree. This individual would be typed as homozygous with conventional STR typing methods, but with the Ibis STR assay he was identified as heterozygous at this locus, with allele 15 (G→A) derived from the primary pedigree.

Trio samples potentially having germline mutations transmitted from parent to offspring were identified with conventional STR typing methods by our UNTHSC collaborators. A panel of these samples was analyzed in a blinded fashion with the Ibis

STR assay using a subset of loci showing the highest frequencies of SNPs: D13S317, D21S11, D3S1358, D5S818, D7S820, D8S1179, and vWA. Results were curated to remove sample sets where relatedness of a parent to the child was disproven, and where there was no evidence of a germline mutation among the loci that were analyzed. The remaining family trios showed length-varying mutations consistent with the results obtained with conventional typing methods. In most cases the parental origin of the mutation was evident with conventional methods, but there were several instances where the origin was evident only with the additional discrimination afforded by the detection of SNPs in the STR loci (e.g., groups 2, 52). Results appear in Table 35.

Positive and negative controls

Table 17. Genotypes of commercially available DNA.

	Promega 9947A DNA		HL-60 (ATCC P/N CCL-240)	
locus	Conventional STR typing[1]	Ibis STR	Conventional STR typing[2]	Ibis STR
AMEL	X, ---	X, ---	X, ---	X, ---
CSF1PO	10, 12	10, 12	13, 14	13, 14
D13S317	11, ---	11, ---	8, 11	8, 11 (A->T)
D16S539	11, 12	11, 12	11, ---	11, ---
D18S51	15, 19	15, 19	14, 15	14, 15
D21S11	30, ---	30, ---	29, 30	29, 30 (A->G)
D3S1358	14, 15	14 (G->A), 15 (G->A)	16, ---	16 (G->A), ---
D5S818	11, ---	11, ---	12, ---	12, ---
D7S820	10, 11	10 (T->A), 11	11, 12	11, 12
D8S1179	13, ---	13, 13 (A->G)	12, 13	12, 13 (A->G)
FGA	23, 24	23, 24	22, 24	22, 24
THO1	8, 9.3	8, 9.3	7, 8	7, 8
TPOX	8, ---	8, ---	8, 11	8, 11
vWA	17, 18	17, 18	16, ---	16, ---

[1] As specified for Control DNA 9947A in the Applied Biosciences Identifiler kit.
[2] Timken *et al.*, 2005. A duplex real-time qPCR assay for the quantification of human nuclear and mitochondrial DNA in forensic samples: Implications for quantifying DNA in degraded samples. J. Forensic Sci. 50(5):1044-1060.

Two negative controls have been created in the course of the STR assay development. The No Addition Control is a virtual sample in which no sample is added to the sample wells, and the plate seal is not pierced. After amplification and analysis any detection is suggestive of an intrinsic DNA contaminant within the reaction. Without sample addition the reaction volume is 35 μL rather than the typical 40 μL volume, but amplification in this reduced volume was confirmed with the accurate analysis of 0.5 μL volumes of concentrated DNA samples. In addition, a Negative Control has been developed which consists of Ibis Primer Dilution Buffer aliquoted and dispensed in plasticware treated to remove trace contaminating human DNA. This control is meant for the evaluation of the sample loading process. Genotypes were determined for

human DNA 9947A (Promega) and the cell line HL-60 (DNA extracted from the ATCC cell line, P/N CCL-240), both of which are commercially available for use as a positive control in the assay. Results appear in Table 17.

The purified human DNA samples used for the developmental validation of the assay subsequently have been used internally for quality control during the fabrication of kitted plates. From 12-24 QC samples, as well as No Addition Controls, are analyzed at critical points during the kitting process. Kitting advances to the next step only with favorable results for a set of QC metrics encompassing profile determinations, allelic balance, interlocus balance, signal amplitude, and the lack of contaminating DNA.

Allelic balance and interlocus balance

Allelic balance and interlocus balance were characterized using data generated in the concordance study. For both assays 1 ng per reaction huDNA was analyzed. Allelic balance was calculated for all heterozygous loci as the amplitude ratios of the smaller allele divided by the larger allele. Table 18 shows allelic balance of the 53 samples genotyped with the Identifiler and Ibis STR assays. Note that the Ibis STR assay does not genotype the D19S433 or the D2S1338 loci. The Identifiler assay showed average allelic balances greater than 1.0 for all loci, indicating that the smaller allele of a heterozygous sample was more abundant than the larger allele on average. This was true for the Ibis STR assay as well; relative to the Identifiler assay, the Ibis STR assay gave greater average allelic balance with wider variation about the mean.

Table 18. Allelic balance for the Applied Biosystems Identifiler assay and the Ibis STR assay.

Locus	Identifiler	Ibis STR
AMEL	1.12 ± 0.26	0.94 ± 0.23
CSF1PO	1.02 ± 0.17	1.20 ± 0.14
D13S317	1.09 ± 0.17	1.31 ± 0.25
D16S539	1.10 ± 0.16	1.22 ± 0.42
D18S51	1.12 ± 0.15	1.40 ± 0.29
D19S433	1.09 ± 0.13	na
D21S11	1.07 ± 0.15	1.27 ± 0.24
D2S1338	1.12 ± 0.15	na
D3S1358	1.08 ± 0.10	1.16 ± 0.18
D5S818	1.07 ± 0.13	1.16 ± 0.24
D7S820	1.02 ± 0.11	1.23 ± 0.35
D8S1179	1.05 ± 0.13	1.18 ± 0.23
FGA	1.11 ± 0.24	1.28 ± 0.28
TH01	1.03 ± 0.14	1.22 ± 0.22
TPOX	1.03 ± 0.13	1.15 ± 0.14
vWA	1.06 ± 0.14	1.30 ± 0.26

For the calculation of interlocus balance, the amplitudes associated with the allele determinations of a particular locus were divided by the sum of all amplitudes in the multiplexed reaction. The Identifiler assay is a 16-plex reaction, and so the expected signal proportion for each locus would be 1/16th of the sum of all allele amplitudes of the reaction. The Ibis STR assay has five 3-plex reactions. In this case the expected signal proportion for a locus would be 1/3rd of the summed amplitudes of the reaction, and there would be 5 independent interlocus balance measures, one for each of the five multiplexed reactions. Interlocus balance observed with analysis of the 53 sample set are shown in Tables 19 and 20. The expected signal proportion for the Identifiler assay is 0.0625. The proportion of the total reaction amplitude shown by each locus was

noted for each of the 53 samples and averaged; in Table 19 it can be seen that average values ranged from 0.01 – 0.08. For the Ibis STR assay there were five interlocus balance measures, one for each of the five multiplexes appearing in wells A-E of the assay. For these triplexes the expected signal proportion is 0.33. Interlocus balance was calculated for these wells as described and averaged across the 53 samples. Averages ranged from 0.27 – 0.36 (Table 20).

Table 19. Interlocus balance for the ABI Identifiler assay.

Locus	Interlocus Balance
AMEL	0.04 ± 0.00
CSF1PO	0.05 ± 0.00
D13S317	0.08 ± 0.01
D16S539	0.08 ± 0.00
D18S51	0.04 ± 0.00
D19S433	0.08 ± 0.01
D21S11	0.05 ± 0.00
D2S1338	0.06 ± 0.01
D3S1358	0.09 ± 0.01
D5S818	0.03 ± 0.00
D7S820	0.04 ± 0.00
D8S1179	0.08 ± 0.01
FGA	0.01 ± 0.00
TH01	0.08 ± 0.01
TPOX	0.06 ± 0.00
vWA	0.05 ± 0.01

The potential for differential amplification among loci within the multiplexed reactions was evaluated using the data from the sensitivity study, where four DNA samples were diluted and analyzed at 50–500 pg per well in replicates of fifty. Locus amplitudes were determined by averaging the signal intensities of the two PCR strands of an allele for homozygous samples, or summing the average signal intensities of both alleles for heterozygous samples. Allelic balance of heterozygous samples was calculated using the ratio of average signal intensities of the smaller allele *versus* the larger allele. Interlocus balance was calculated as the ratio of the locus amplitude in a reaction *versus* the sum of all locus amplitudes for that well. Some detections were missed when low levels of DNA were analyzed, and in these instances the affected well was excluded from the calculation of interlocus balance. Values were averaged across the levels of replication of the experiment and plotted (Figure 22). On average the amplitudes and balance measures were consistent across the range of DNA input levels. Locus amplitudes showed a modest trend downward with lower levels of input DNA, as would be expected. Additionally the replicate allelic balance values showed more dispersion with low levels of DNA, which also would be expected due to sampling effects. However, taken together these data show no evidence of a systematic bias in amplification of the component loci in the multiplexed reactions, even with challenging levels of input DNA.

Table 20. Interlocus balance for the Ibis STR assay

Assay Well	Locus	Interlocus Balance
A	CSF1PO	0.35 ± 0.04
	D3S1358	0.35 ± 0.02
	vWA	0.29 ± 0.03
B	D13S317	0.30 ± 0.04
	D16S539	0.32 ± 0.03
	THO1	0.36 ± 0.04
C	AMEL	0.35 ± 0.02
	D8S1179	0.27 ± 0.03
	TPOX	0.36 ± 0.02
D	D3S1358	0.32 ± 0.02
	D5S818	0.34 ± 0.04
	D7S820	0.33 ± 0.04
E	D16S539	0.35 ± 0.03
	D5S818	0.30 ± 0.03
	vWA	0.33 ± 0.03

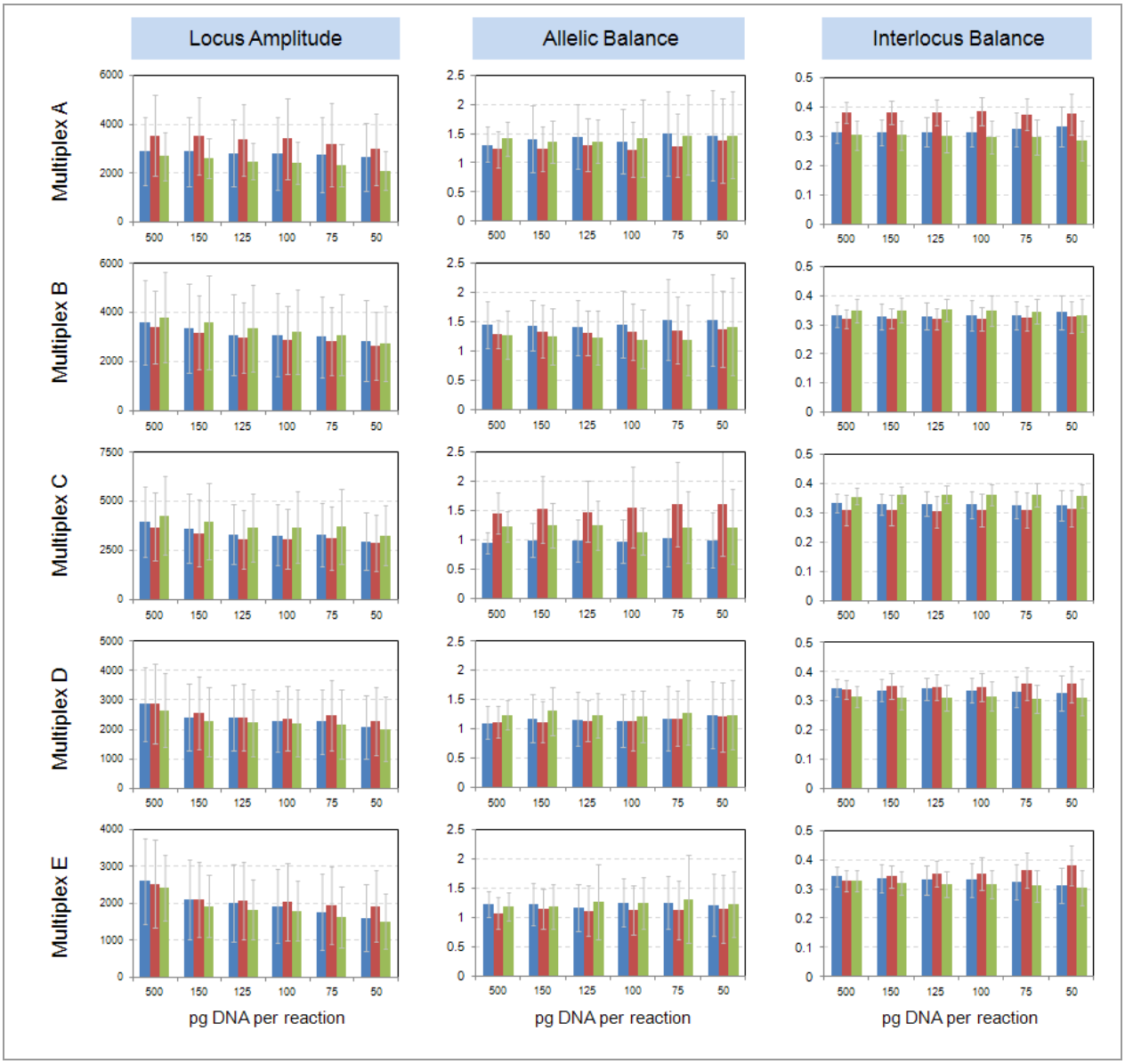


Figure 22. Coamplification of the component loci in multiplexed reactions. Four DNA samples were diluted and analyzed at 50 – 500 pg per well in replicates of fifty. Locus amplitudes, allelic balance, and interlocus balance were averaged across the levels of replication of the experiment for each of the three component loci of each of the five multiplexed reactions in the assay. Plots are colored to indicate the loci within each multiplex as illustrated in the tabulated color scheme.

Multiplex	locus 1	locus 2	locus 3
A	CSF1PO	D3S1358	vWA
B	D13S317	D16S539	TH01
C	AMEL	D8S1179	TPOX
D	D3S1358	D5S818	D7S820
E	D16S539	D5S818	vWA

Mixture studies

Mixture analysis was performed using samples created with known quantities of purified human DNA samples. Sample mixtures were created with samples having an assortment of unique alleles relative to one another. To define the range of mixture proportions where a sample can be identified as having multiple source DNA, sample mixtures with a wide range of source proportions were created using the samples described in Table 21. Source-unique alleles are highlighted, but the total number of

source-unique alleles detected in the assay will be greater because of the redundant detection of some of the assay loci (Figure 15). Samples were mixed in mass ratios of 20:1 through 1:20, with the dominant component of the mixture held constant at 2 ng. Samples were analyzed using 2 ng of the dominant source of the mixture per well of the assay. The detection of alleles unique to either of the two DNA sources was tracked, and the results across the dilution series are shown in Figure 23. Alleles of the minor source in the mixture were detected even at the extremes of the mixture series, at ratios of 20:1 and 1:20. However, only in the central range of the dilution series do the detections show a dose-response where the number of detections is more directly related to the proportional amount of the DNA. This range of proportions, from 5:1 through 1:5, provides a conservative range within which to expect mixture detections with the Ibis STR assay.

Table 21. STR profile of huDNA samples used to create sample mixtures for the analysis of mixture identification.

locus	Ibis STR Profile			
	huDNA-15		huDNA-24	
AMEL	X	Y	X	Y
CSF1PO	10	12	11	--
D13S317	9	11	8	12 (A->T)
D16S539	12	13	11	--
D18S51	16	--	14	16
D21S11	29	32 (A->G)	29	30 (A->G)
D3S1358	15 (2G->2A)	17	14 (G->A)	--
D5S818	12	13 (G->C)	11	12
D7S820	11	--	10 (T->A)	12
D8S1179	13 (A->G)	16 (A->G)	10	14 (A->G)
FGA	24	--	20	22
THO1	9	9.3	6	7
TPOX	11	--	8	--
vWA	16	--	17	--

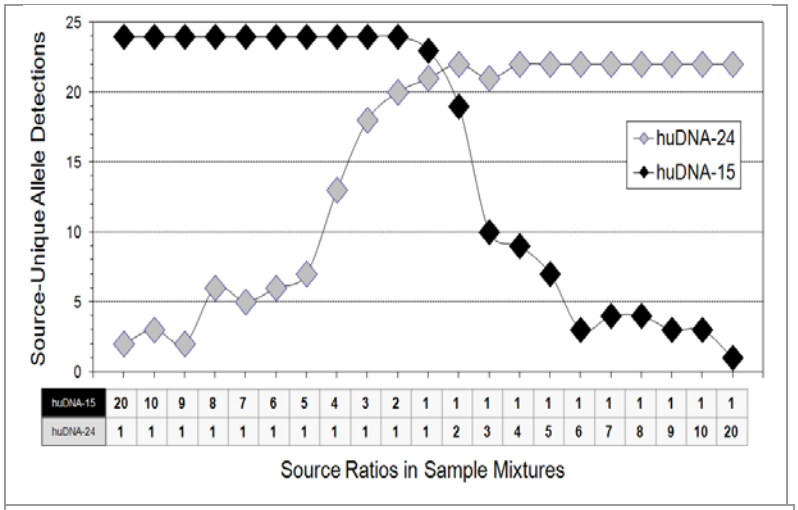


Figure 23. Identification of multisource DNA samples at different mixture ratios. Two human DNA samples having the STR profiles listed in Table 21 were mixed in the indicated ratios. In all cases the major source was held at 2 ng and the minor source was added at the indicated proportion. The equivalent of 2 ng of the major source DNA was analyzed in the Ibis STR assay, and detection of source-unique alleles was tracked across the sample set and plotted above.

Source attribution in mixed DNA samples is possible, depending on the proportion of the DNA sources comprising the mixture, and the balance of the STR typing assay. A more quantitative mixture analysis was done to evaluate the relationship of source-unique allele amplitudes *versus* their relative concentrations in the mixture. The

Table 22. STR profile of huDNA samples used to create sample mixtures for quantitative analysis of source proportions.

locus	STR Profile			
	huDNA-2		huDNA-46	
AMEL	X	Y	X	Y
CSF1PO	10	12	10	13
D13S317	10	12 (A->T)	8	12 (A->T)
D16S539	11	12	11	13
D18S51	14	15	11	16
D21S11	28	32.2	27	28
D3S1358	13	17	14 (G->A)	15 (G->A)
D5S818	9 (G->T)	11	11	13
D7S820	11	12	8	10
D8S1179	9	11	12 (A->G)	15 (A->G)
FGA	20	25	19	21
THO1	7	10	6	9
TPOX	8	11	8	11
vWA	14 (A->G + 2T->2C)	15 (G->A)	18	19

samples listed in Table 22 were used to create mixtures as described above, but in the series 10:1, 5:1, 2.5:1, 2:1, 1.5:1, 1:1, etc.

An example of allele amplitudes appears in Figure 24, where the deconvolved spectrum from well B of the assay is shown for the 1:2.5 ratio of a mixture of huDNA-2 and huDNA-46. Visually the amplitudes of source-unique alleles trend with the relative proportions of the component human DNA samples in the mixture. Mixture ratios were calculated by noting the amplitudes of the source-unique alleles and taking their ratios. Average responses were quantitative over a range of proportions from 1:2.5 through 2.5:1 and appear in Table 23. On average observed ratios were similar to the expected, suggesting that within this range relative amplitudes vary with source proportions. However, a more extensive characterization of mixtures would be required to evaluate this application of the Ibis STR assay.

Table 23. Expected and observed source-unique allele ratios for a panel of mixed DNA samples.

Expected Ratio	Observed Ratio
1 : 2.5	1 : 2.54 ± 0.070
1 : 2	1 : 2.06 ± 0.52
1 : 1.5	1 : 1.58 ± 0.31
1 : 1	1 : 1.09 ± 0.30
1.5 : 1	1.50 ± 0.15 : 1
2 : 1	1.89 ± 0.47 : 1
2.5 : 1	2.22 ± 0.47 : 1

Stability

Plates from the first lot fabricated in the R&D Pilot Manufacturing Lab were frozen and sequestered for an analysis of stability. On a 2 week schedule, duplicate plates were thawed and loaded with huDNA-24 and huDNA-46 in duplicate, diluted in the same series that was used in the analysis of sensitivity described above. From 6-8 allele detections were expected across the levels of replication of the test, depending on the level of heterozygosity of the sample. Missed detections were tallied, and results through week 44 of the ongoing 52-week study are summarized in Figure 25. Note that PP4451 replaced PP4971 for the detection of D21S11 after this study was initiated. A parallel stability study has been instituted with PP4451, and the schedule for these plates is on a 22 week lag relative to the primary stability study. Full detections have been seen at the 500 pg per well level throughout the study to date, single missed detections were seen at five timepoints at the 150 pg per well level, and 0-3 missed detections were seen at the remaining time points. The evaluation will continue through the 52-week time period, and there are sufficient sequestered plates to extend the study further.

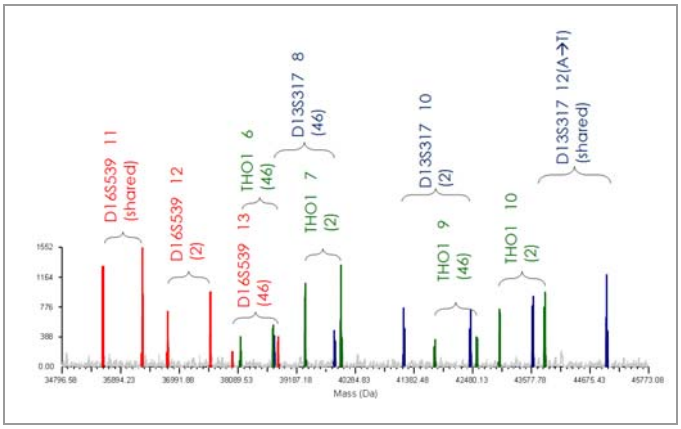


Figure 24. Deconvolved spectrum of a 1 : 2.5 mixture analyzed with multiplex B of the Ibis STR assay. Both PCR strands of the allele products for each of the three loci targeted in this reaction are shown: D16S549 (red), THO1 (green), and D13S317 (blue). Sources of the individual products are indicated as shared, from huDNA-2 (2), or from huDNA-46 (46).

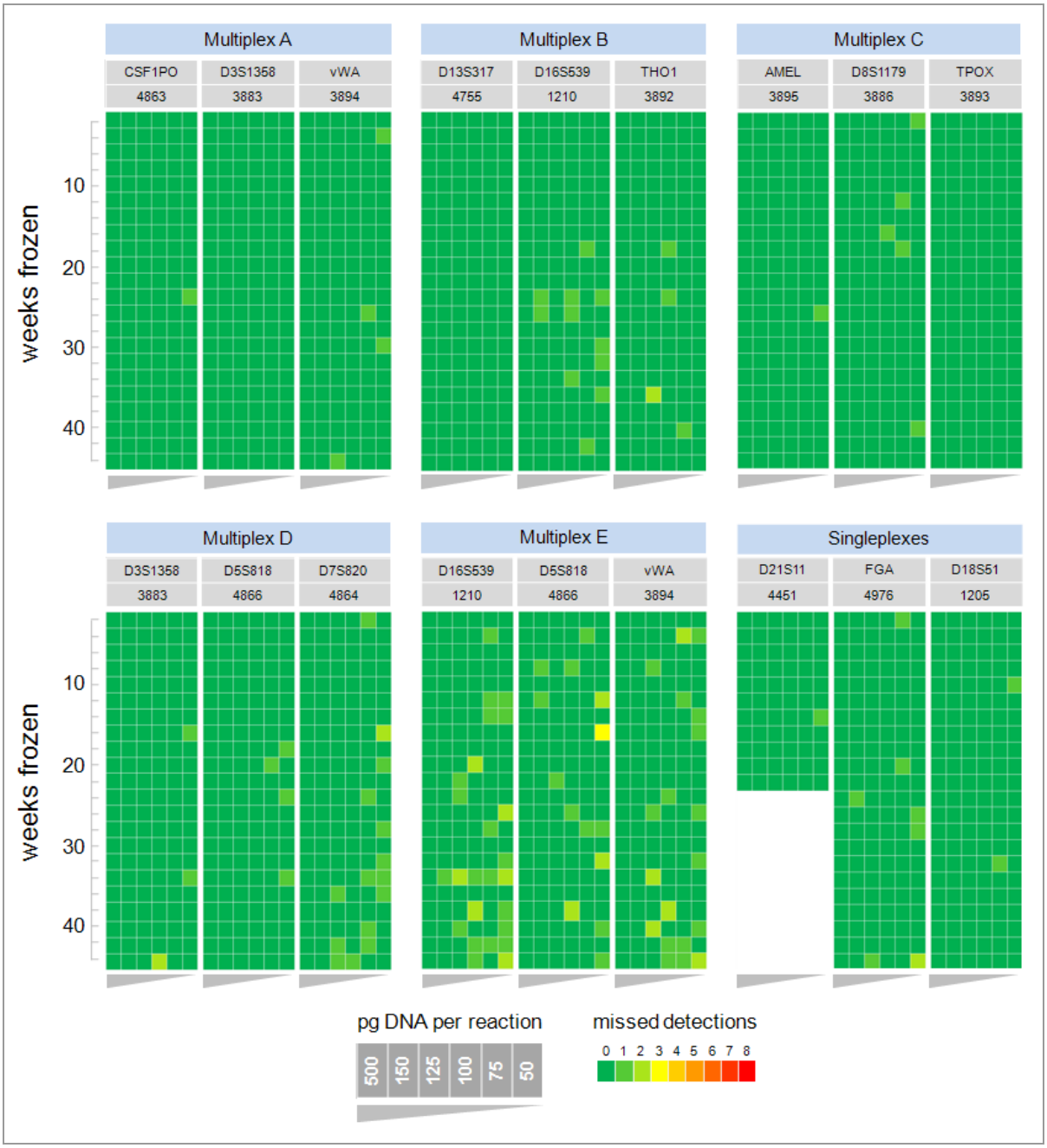


Figure 25. Stability of prefabricated STR assay plates. STR assay plates were kitted in the Ibis Pilot Manufacturing Suite and frozen at -20°C. At two week intervals two plates were thawed, loaded with sample, amplified, and analyzed with the T5000 mass spectrometer. A dilution series of 50–500 pg DNA per well were prepared for each of two human DNA samples, and run in duplicate. From 6-8 allele detections were expected across the levels of replication of the test, depending on the level of heterozygosity of the sample. Missed detections were tallied and summarized above, with the color gradient indicating the number of missed detections at a given timepoint and DNA input level. Note that the D21S11 primer pair PP4451 was set up separately at a 22 week lag relative to the remainder of the assay, since the original D21S11 primers were found to have suboptimal stability once the stability study was underway.

Specific Aim 2: Develop an ESI-MS assay for the SWGDAM-recommended Y-STR markers.

Background material:

Following our approach for developing an automated assay for autosomal STR markers, we proposed to develop a multiplexed PCR reaction panel to target the Y-STR loci that have become the core forensic standards. This effort focuses upon the minimal haplotype set, namely DYS393, DYS19, DYS391, DYS389I/II, DYS390, DYS385a/b and DYS392[23], along with the widely used markers DYS437, DYS438 and DYS439[23, 63]. Information required to perform Y-STR analyses fits directly into our current allele-based genotyping system. The proposed plan was to cover all loci in four reactions. Although we strive to reduce the number of reactions as much as possible, we are constrained by the limits of signal distribution and spectral congestion that result from analyzing large amplicons using ESI-MS.

We have experience analyzing PCR products >250 bp in length, but signals in ESI-MS become distributed across many closely-spaced charge states, which can cause a problem multiplexing very large amplicons with other products due to signal collisions. For example, DYS385a/b has a large product length range (241-324 bp for the shortest primer set listed in STRbase)[47, 64-67]. There is substantial length contributed to the PCR product by an extended A/G region upstream of the 'GAAA' repeat. We proposed to take advantage of a distinct pattern of 'A' and 'G' present in this region to create a primer binding site to reduce the product length range to 109-193 bp.

Another locus with a large product size range is DYS389I/II, which produces a small product (DYS389I) along with a larger product by virtue of duplicated binding sites for the forward primer[67]. We proposed to attempt amplifying the two regions separately. By utilizing a 3´ end difference in the forward primer binding region ('TGTG' in the second region as opposed to 'TATC' in the first region) to favor formation of the shorter DYS389I product. The same forward primer with the first region 'TATC' at the 3' end would be used along with a reverse primer extending back upstream of the second forward primer site to favor formation of the first part of DYS389II (excluding the repeat region of DYS389I). These pairs were to be included in different multiplexes (they cannot be put together).

New work under the current award:

2.1 Development of a multiplex Y-STR assay

For the ten Y-STR loci DYS393, DYS19, DYS319, DYS439, DYS389I/II, DYS438, DYS390, DYS385a/b, DYS392 and DYS437, multiple primer pairs were selected for each locus for testing in the same buffer conditions as those used in the Ibis mtDNA tiling assay and the currently planned STR assay (Table 24). All primer pairs were initiated with a 'T' to minimize non-templated adenylation by *Taq* polymerase.

The published structures for each cataloged allele were used to compile a database of reference allele products according to the existing allele nomenclature for all primer pairs listed in Table 24. Locus references and published allele structures were available for the targeted loci through the STRBase database[47]. Testing of Y-STR primers was initiated in the same buffer conditions used for autosomal STRs: each 40 μl reaction contained 10 mM Tris-Cl, 75 mM KCl, 1.5 mM $MgCl_2$, 400 mM betaine, 200 μM each of dATP, dCTP, and dTTP (BioLine), 200 μM ^{13}C-enriched dGTP (Cambridge Isotope Laboratories), 1.5 U/reaction Immolase (BioLine). All primers were tested in duplicate in single primer pair reactions using 1 ng of template DNA (male blood sample

Table 24. Preliminary primer pairs selected for testing against Y-STR markers. Multiple primer pairs were selected to each locus to increase multiplexing choices. The preliminary groupings for loci are shown in the left column. The final panel would occupy four reactions.
*, ¥ The DYS389I locus and 5' side of DYS389I/II were targeted separately by exploiting a four-base region between the two repeat regions that allows specific targeting of each of the two repeated forward primer binding sites for DYS389I/II.
£ The product for DYS385a/b was minimized using a reverse primer placed in an A-G region with a unique pattern that brings the primer near the repeat region.
± If separately amplifying the components of DYS385I/II did not work, or proves to be unreliable over many samples, we will consider running this primer pair in single-plex.

Target Panel	Locus	Ibis Primer Pair Number	Primer Pair Name	Product Length Range	Primer Sequences
1	DYS390	4588	DYS390_AC011289_11029_11210	154-198	TGGGCCCTGCATTTTGGTAC TCATTGCAATGTGTATACTCAGAAACAAG
		4589	DYS390_AC011289_11022_11206	157-201	TCATTTTTGGGCCCTGCATTTTG TGCAATGTGTATACTCAGAAACAAGGAAAG
		4590	DYS390_AC011289_11029_11206	150-194	TGGGCCCTGCATTTTGGTAC TGCAATGTGTATACTCAGAAACAAGGAAAG
		4591	DYS390_AC011289_11034_11201	140-184	TCTGCATTTTGGTACCCCATAATATATTC TGTGTATACTCAGAAACAAGGAAAGATAGATA
	DYS391	4592	DYS391_G09613_18_181	149-181	TCCCTTCATTCAATCATACACCCATAT TGCATAGCCAAATATCTCCTGGG
		4593	DYS391_G09613_23_137	99-131	TCATTCAATCATACACCCATATCTGTCTG TCAATTGCCATAGAGGGATAGGTAGG
		4594	DYS391_G09613_23_142	104-136	TCATTCAATCATACACCCATATCTGTCTG TGCAAGCAATTGCCATAGAGG
		4595	DYS391_G09613_26_123	82-114	TTCAATCATACACCCATATCTGTCTGTC TGGATAGGTAGGCAGGCAGATAG
	DYS392	4596	DYS392_AC011745_97244_97358	94-127	TCCAAGCCAAGAAGGAAAACAAA TCAACCTACCAATCCCATTCCTT
		4597	DYS392_AC011745_97256_97363	87-120	TGGAAAACAAATTTTTTCCTTGTATCACCA TCCATTAAACCTACCAATCCCATTCC
		4598	DYS392_AC011745_97249_97362	93-126	TCCAAGAAGGAAAACAAATTTTTTCCTTG TCATTAAACCTACCAATCCCATTCCTTAG
		4599	DYS392_AC011745_97237_97362	105-138	TGTTATTTAAAAGCCAAGAAGGAAAACAAA TCATTAAACCTACCAATCCCATTCCTTAG
	DYS393	4600	DYS393_AC006152_21087_21211	113-145	TAATGTGGTCTTCTACTTGTGTCAATAC TGAACTCAAGTCCAAAAAATGAGGTATGTC
		4601	DYS393_AC006152_21089_21212	112-144	TGGTGGTCTTCTACTTGTGTCAATAC TGGAACTCAAGTCCAAAAAATGAGG
		4602	DYS393_AC006152_21090_21206	105-137	TGTGGTCTTCTACTTGTGTCAATACAGATAG TCAAGTCCAAAAAATGAGGTATGTCTCATAG
		4603	DYS393_AC006152_21092_21203	100-132	TGGTCTTCTACTTGTGTCAATACAGATAG TGTCCAAAAAATGAGGTATGTCTCATAG
	DYS389I*	4585	DYS389I_AC004617_126008_126167	148-180	TCCAACTCTCATCTGTATTATCTATGTATCTG TCACAGTTATCCCTGAGTAGTAGAAGAATG
		4586	DYS389I_AC004617_126008_126107	88-120	TCCAACTCTCATCTGTATTATCTATGTATCTG TAGATAGATTGATAGAGGGAGGGATAGATAG
2	DYS389II-1¥	4587	DYS389II-1_AC004617_125888_126021	106-146	TCCAACTCTCATCTGTATTATCTATGTGTG TGATGAGAGTTGGATACAGAAGTAGGTATAATG
	DYS437	4604	DYS437_AC002992_42957_43139	171-187	TGTGAGTGCATGCCCATCC TGACCCTGTCATTCACAGATGATATAGATAG
		4605	DYS437_AC002992_42956_43127	160-176	TCGTGAGTGCATGCCCATC TCACAGATGATATAGATAGATAGATAACCACAGA
		4606	DYS437_AC002992_42951_43127	165-181	TATGGGCGTGAGTGCATGC TCACAGATGATATAGATAGATAGATAACCACAGA
		4607	DYS437_AC002992_42949_43096	136-152	TCTATGGGCGTGAGTGCATG TGGTAAATATCATTCATAGATAAGTAGATAGACATC
		4608	DYS437_AC002992_42956_43087	120-136	TCGTGAGTGCATGCCCATC TCGTTCATAGATAAGTAGATAGACATCATTCAC
	DYS438	4609	DYS438_AC002531_129796_129952	137-177	TAGTGGGGAATAGTTGAACGGTAA TGGAGGTTGTGGTGAGTCGAG
		4610	DYS438_AC002531_129798_129911_2	94-134	TTGGGGAATAGTTGAACGGTAAACAG TCTGGGCAACAAGAGTGAAACTC
		4611	DYS438_AC002531_129788_129914	107-147	TCCAAAATTAGTGGGGAATAGTTGAACG TAGCCTGGGCAACAAGAGTG
		4612	DYS438_AC002531_129798_129919_2	102-142	TTGGGGAATAGTTGAACGGTAAACAG TATTTCAGCCTGGGCAACAAGAG
	DYS439	4613	DYS439_AC002992_91258_91396	123-143	TAGATACATAGGTGGAGACAGATAGATGAT TGGCCTGGCTTGGAATTCTTTT
	DYS439	4614	DYS439_AC002992_91262_91393	116-136	TACATAGGTGGAGACAGATAGATGATAAATAG TCTGGCTTGGAATTCTTTTACCCATC
	DYS439	4615	DYS439_AC002992_91254_91390	121-141	TAGATAGATACATAGGTGGAGACAGATAGATG TGCTTGGAATTCTTTTACCCATCATCTC
	DYS439	4616	DYS439_AC002992_91262_91390	113-133	TACATAGGTGGAGACAGATAGATGATAAATAG TGCTTGGAATTCTTTTACCCATCATCTC
3	DYS19	4578	DYS19_AC017019_118941_119119	171-207	TCACTATGACTACTGAGTTTCTGTTATAGTG TCCATCTGGGTTAAGGAGAGTGTC
		4579	DYS19_AC017019_118947_119118	164-200	TGCCTACTGAGTTTCTGTTATAGTGTTTTTT TCATCTGGGTTAAGGAGAGTGTCAC
		4580	DYS19_AC017019_118947_119113	159-195	TGACTACTGAGTTTCTGTTATAGTGTTTTTT TGGGTTAAGGAGAGTGTCACTATATC
4	DYS385a/b£	4581	DYS385-A-B_AC022486_29394_29615	206-290	TCAACAAAGAAAAGAAATGAAATTCAGAAAGG TCCAATTACATAGTCCTCCTTTCTTTTTCTC
		4582	DYS385-A-B-2_AC022486_29491_29615	109-193	TGAAAGAGAAAGAGGAAAGAGAAAGAAAGG TCCAATTACATAGTCCTCCTTTCTTTTTCTC
Backup	DYS389I-II±	4584	DYS389I-II_AC004617_125888_126106	199-239	TCCAACTCTCATCTGTATTATCTATGTGTG TGATAGATTGATAGAGGGAGGGATAGATAG

SC35495 from SeraCare, Inc.). Thermocycling consisted of 96 °C, 10 min, 40 cycles of [96 °C, 25 sec, 56 °C, 1.5 min, 72 °C, 40 sec], 72 °C, 4 min, 4 °C hold. The first test of the Y-STR primers suggested that there was at least one primer pair per locus that was likely to perform sufficiently to carry forward to a final assay. A qualitative assessment of initial primer pair performance is shown in Table 25 (raw data not shown). The strategy used to shorten the products from DYS385a/b to a maximum size of less than 200 bp appeared promising (Figure 26, A.). Also, the strategy to split DYS389I/II into two separate, manageable products appeared to be successful (Figure 26, B.). One primer pair for each locus was then chosen to carry forward for multiplexing. Note that the numerical sum of the alleles assigned for DYS389I and DYS389II-1 (13 and 16, respectively in Figure 26B) will be

Table 25. Qualitative assessment of primer pair performance for candidate Y-STR primer pairs. Green highlighting indicates primer pairs that were carried forward for multiplexing (one per locus). Light green indicates backup primmer pairs that may be tested as replacements if any primer pairs show sample-dependent or sensitivity problems. Yellow highlighting indicates sub-optimal performance that might be addressed by slight modification of the primers. Red highlighting indicates primer pairs that were dismissed as possible choices.

pp	Target	Continue	Backup	Discontinue	Comments - qualitative result description
4578	DYS19			x	Low signal - Barely primes - not obvious why
4579	DYS19	x			Strong signal
4580	DYS19		x		Good, but more adenylation than 4579
4581	DYS385a/b		x		Interpretable, but large product diminshes data quality
4582	DYS385a/b	x			Interpretable signal. Smaller product than 4581
4584	DYS389II		x		Highly adenylated on forward strand
4585	DYS389I		x		Look OK - product somewhat large
4586	DYS389I	x			Strong signal, product smaller than 4585
4587	DYS389II-1	x			Half of DYS389I/II, excluding DYS389I. Good signal, but adenylation on forward strand
4588	DYS390		x		Heavy adenylation of reverse strand.
4589	DYS390		x		Good signal, adenylation a little high on reverse strand.
4590	DYS390		x		Heavy adenylation of reverse strand.
4591	DYS390	x			Good signal, and produces smallest products for DYS390 primers
4592	DYS391		x		Signal OK. Looks clean
4593	DYS391		x		Good signal, small products, a little adenylation
4594	DYS391	x			Good signal, small products, a little adenylation
4595	DYS391		x		Good signal, but there may be a dimer along with the product
4596	DYS392		x		Very strong, clean signal, both up and down stutter
4597	DYS392	x			Very strong, clean signal, both up and down stutter
4598	DYS392		x		Very strong, clean signal, both up and down stutter
4599	DYS392			x	Weaker, messier than other DYS392 primer pairs
4600	DYS393		x		Two products, one with G->C SNP
4601	DYS393		x		One product, strong adenylations
4602	DYS393	x			Two products, one with T->C SNP -- strands a little unbalanced, but best overall signal and lowest adenylation for DYS393
4603	DYS393		x		Two products, one with T->C SNP
4604	DYS437		x		OK, but a little messy, some adenylation
4605	DYS437		x		OK, but a little messy, some adenylation
4606	DYS437		x		OK, but a little messy, some adenylation
4607	DYS437		x		Better, but a little messy, some adenylation
4608	DYS437	x			Looks best for DYS437
4609	DYS438			x	Low signal, messy, some adenylation, high baseline
4610	DYS438		x		Signal a little low, a little messy, some adenylation, high baseline
4611	DYS438	x			Better, but signal still a little low, a little messy, some adenylation, high baseline
4612	DYS438			x	Low signal, messy, some adenylation, high baseline
4613	DYS439		x		Good signal, adenylation on forward strand
4614	DYS439		x		Decent signal, adenylation on forward strand
4615	DYS439	x			Great signal, adenylation very low
4616	DYS439		x		Good signal, a little adenylation.

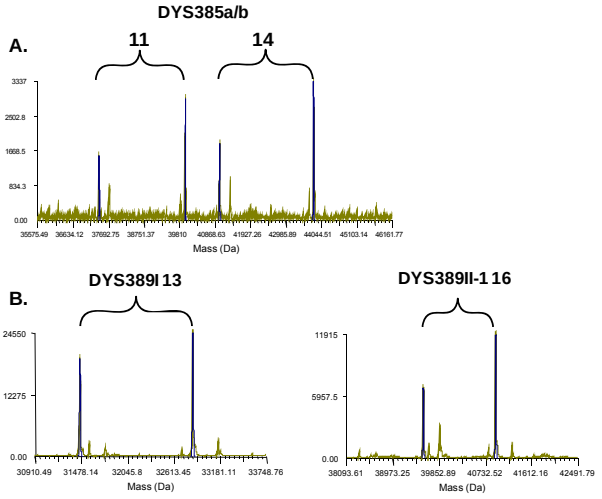


Figure 26. Testing of primer pair targets designed to shorten products for DYS385a/b and to split DYS389I/II into two separate products. In A., a primer pair designed to exploit specificity in a non-repeating, but low-complexity A/G-rich region near the repeat region successfully shortens the product to be clearly resolvable in the mass spectrometer. The region (not shown here) corresponds to coordinates 126,828-126,866 from GenBank accession AC022486.4, but primers are designed on the reverse complement of the reported sequence. Figures demonstrating the priming region are available upon request. In B., Two primer pairs split the DYS389I-II locus into DYS389I and the 5′ half that we have labeled as DYS389II-1 (first half of DYS389II). Figures demonstrating the approach are available upon request.

equal to the DYS389II allele assignment made by a conventional typing kit such as Y-Filer (e.g., the sample shown in Figure 26, B. will generate an allele 29 for DYS389II). This has been demonstrated by concordance testing with Y-Filer and is discussed further on in this report.

An additional allele was amplified with three of four initial primer pairs for DYS393. Two of these primer pairs (4602 and 4603) clearly produced an allele 13 and an additional product with a base composition consistent with an allele 13 with a T→C SNP in it (Figure 27). One primer pair (4600) produced an allele 13 and a product consistent with allele 13 with a C→G SNP in it (not shown). The other primer pair (4601) produced only one product (allele 13). The initial primer pair panel chosen to move forward with was intended to exploit the additional discriminating information that may be revealed by the presence of an additional allele at DYS393. The hypothesis Was that the locus may have been duplicated and that the individual used for testing had a SNP in one of the two loci. Conventional typing would not detect this. Testing of population samples (see below) showed this hypothesis to be incorrect, as two alleles were produced in all samples and many of them were different lengths. The second allele contained a T→C SNP in every case, but appeared at lengths consistent with DYS393 alleles 12, 13, 14, 15 and 16. The second allele is a homologous locus from the X-chromosome[68, 69], and the panel was modified by switching to primer pair 4601 in order to exclude the X-chromosome homolog (genetic sequences not shown, but sequences and demonstrative figures available upon request).

Multiplexing tests were initiated using the primer pairs and concentrations shown in Table 26. The same buffer conditions and thermocycling were used as described above for single-plex testing. Primer pairs in multiplexes were used at equal concentrations designed to total 1.6 μM for all primers combined (average of 200 nM per primer for a 4-plex, or 160 nM per primer for a 5-plex). Blood sample SC35495 was

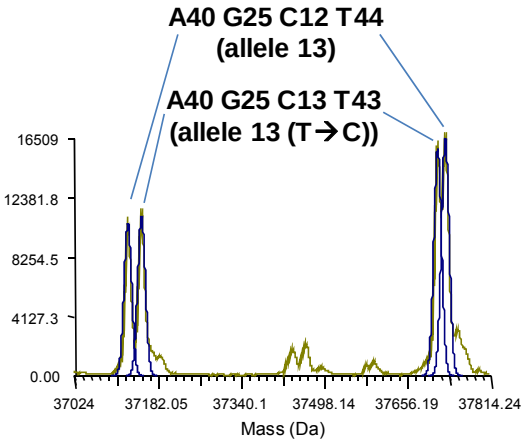


Figure 27. Two products were amplified for DYS393. The example above shows two products generated from a male DNA template using a single primer pair targeting DYS393.

Table 26. Primer pairs and concentrations used for initial multiplex testing of Y-STR primers.

Reaction	Primer pair	Locus	Conc (nM)
Multiplex 1	4586	DYS389I	160
	4597	DYS392	160
	4594	DYS391	160
	4602	DYS393	160
	4591	DYS390	160
Multiplex 2	4587	DYS389II-1	200
	4611	DYS438	200
	4615	DYS439	200
	4608	DYS437	200
Single-plex 1	4579	DYS19	250
Single-plex 2	4582	DYS385a/b	250

Table 27. Additional Y-STR primer pairs screened for incorporation into the Ibis Y-STR assay.

Ibis primer pair number	Locus	Primer pair name	Primer sequences
4902	DYS448	DYS448_AC025227_17214_17445	TGAGACAGAAAGGGAGATAGAGACATGG TATTTCTGGCCGGTCTGGAAATTTATCTC
4903	DYS456	DYS456_AC010106_14130_14252	TGGTCTGTTGTGGGACCTTGTG TAGGGTTCTCTAGAGGGACAGAACTAATGG
4904	DYS456	DYS456_AC010106_14133_14252	TCTGTTGTGGGACCTTGTGATAATGT TAGGGTTCTCTAGAGGGACAGAACTAATG
4905	DYS456	DYS456_AC010106_14124_14260	TGCAGATGGTCTGTTGTGGGAC TGATGTATTAGGGTTCTCTAGAGGGACAG
4926	DYS456	DYS456_AC010106_14133_14249	TCTGTTGTGGGACCTTGTG TGTTCTCTAGAGGGACAGAACTAATG
4927	DYS456	DYS456_AC010106_14135_14250	TGTTGTGGGACCTTGTGATAATGT TGGTTCTCTAGAGGGACAGAACTAATG
4928	DYS456	DYS456_AC010106_14125_14257	TCAGATGGTCTGTTGTGGGAC TGTATTAGGGTTCTCTAGAGGGACAG
4929	DYS456	DYS456_AC010106_14140_14279	TGGGACCTTGTGATAATGTAAGATAGA TGCCCAAAACTTCTTAAACTGATGTATTAG
4930	DYS456	DYS456_AC010106_14133_14242	TCTGTTGTGGGACCTTGTGATAATG TAGAGGGACAGAACTAATGGAATATCTATC
4939	DYS456	DYS456_AC010106_14139_14280	TTTGGACCTTGTGATAATGTAAGATAGA TTTTCCAAAACTTCTTAAACTGATGTATTAG
4940	DYS456	DYS456_AC010106_14133_14241	TTTGTTGTGGGACCTTGTGATAATG TTTGGGACAGAACTAATGGAATATCTATC
4941	DYS456	DYS456_AC010106_14141_14246	TGGACCTTGTGATAATGTAAGATAG TCTCTAGAGGGACAGAACTAATG
4942	DYS456	DYS456_AC010106_14137_14244	TTGTGGGACCTTGTGATAATGT TCTAGAGGGACAGAACTAATGGA
4943	DYS456	DYS456_AC010106_14138_14246	TGTTGGACCTTGTGATAATGTAAGATAG TCTCTAGAGGGACAGAACTAATG
4944	DYS456	DYS456_AC010106_14140_14242	TGGGACCTTGTGATAATGTAAGATAGA TTGAGGGACAGAACTAATGGAATATCTATC
4906	DYS458	DYS458_AC010902_44001_44152	TGCAGACTGAGCAACAGGAATGAAAC TCTGGCATTACAAGCATGAGCCAC
4907	DYS458	DYS458_AC010902_44002_44144	TCAGACTGAGCAACAGGAATGAAACTC TACAAGCATGAGCCACCACGC
4908	DYS458	DYS458_AC010902_44008_44136	TGAGCAACAGGAATGAAACTCCA TGAGCCACCACGCCCAC
4924	DYS458	DYS458_AC010902_44006_44136	TCTGAGCAACAGGAATGAAACTC TGAGCCACCACGCCCAC
4925	DYS458	DYS458_AC010902_44008_44132	TGAGCAACAGGAATGAAACTCCAATG TCACCACGCCCACCCTC
4909	DYS635	DYS635_AC004772_90964_91115	TCCCCAATCAATGAATGGATAAAGAAAATGTG TCTTGGCTTCTCACTTTGCATAGAATC
4910	DYS635	DYS635_AC004772_90975_91112	TCCCCGGATAAAGAAAATGTGATAGATAGATAG TGGCTTCTCACTTTGCATAGAATCTCTATC
4911	Y-GATA-H4	Y-GATA-H4_G42676_92_272	TCCTGCTGAGGAGAATTTCCAAATTTAAG TCCCCTAACAGGATAAATCAGCTATCTATG
4912	Y-GATA-H4	Y-GATA-H4_G42676_93_238	TCCGCTGAGGAGAATTTCCAAATTTAAG TCCCCTATCTATCTATTCATCCATCTAATCTATCC

tested in duplicate using 1 ng/reaction of DNA. Products in the first multiplex test were not well balanced between loci (not shown). To address this, primer pair concentrations were adjusted iteratively over four more experiments (not shown). In addition, the primer pair for DYS385a/b was modified to increase product yield and reduce adenylation (not shown).

Table 28. Primer pairs used for 13-locus intermediate Y-STR assay.

Reaction	Primer pair	Locus
Multiplex 1	4586	DYS389I
	4597	DYS392
	4594	DYS391
	4601	DYS393
	4591	DYS390
	4924	DYS458
Multiplex 2	4587	DYS389II-1
	4611	DYS438
	4615	DYS439
	4608	DYS437
	4929	DYS456
Single-plex 1	4579	DYS19
Single-plex 2	4692	DYS385a/b

The initial Y-STR primer panel included the minimal haplotypes set plus DYS437, DYS438 and DYS439. This is the same set of loci included in Promega's PowerPlex® Y kit. We have also incorporated the additional loci DYS456, DYS458, DYS448, DYS635 and Y-GATA-H4 (each of these is included in Applied Biosystems' AmpFlSTR® Yfiler™ kit). New primer pairs synthesized and tested are shown in Table 27. It was necessary to screen a large number of primer pairs for DYS456 and DYS458 to find a pair that performed well under the conditions used for the other primer pairs in our multiplexes. This was primarily due to increased annealing stringency required to eliminate non-specific priming on the human genome (data not shown). According to recent data published by Budowle *et. al.*[70], the two most polymorphic Y-STR loci in the three major US populations are DYS385 and DYS458. In addition, the five loci with the highest combined power of discrimination were DYS389II, DYS456, DYS458, DYS439, and DYS385[70]. The two loci DYS456 and DYS458 were not in our initial primer pair panel and we therefore wanted to get those loci incorporated first. Incorporation of additional loci into multiplexes was initiated with primer pair 4924 for DYS458 into multiplex 1 to produce a 6-plex and 4929 for DYS456 into multiplex 2 to produce a 5-plex resulting in an intermediate 13-locus assay layout that performs reasonably well and is capable of running 24 samples per plate (Table 28).

In an effort to finalize markers to be included in a manufactured Y-STR kit, the additional loci DYS635 and Y-GATA-H4 were added to a four-reaction plate layout that

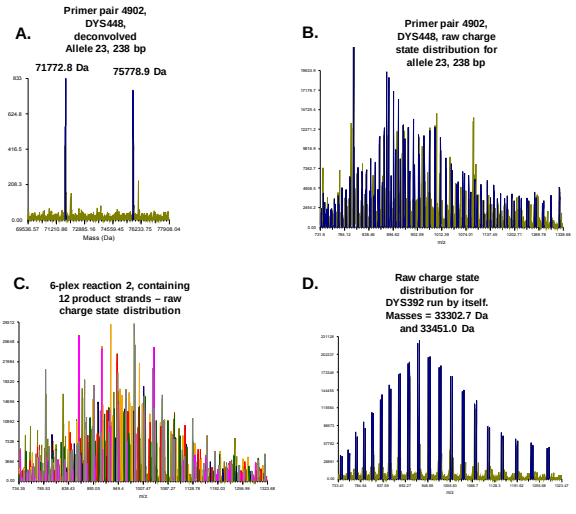


Figure 28. Locus DYS448 run in single-plex reaction produces similar number of charge states as a multiplexed reaction. A.) Deconvolved spectrum of primer pair 4902 for locus DYS448 run with a sample having allele 23. A 238 base pair product is produced that is deconvolved with reasonable data quality as long as the reaction is not multiplexed. B.) The raw charge state distribution contains many peaks spaced close together to produce a congested spectrum with a single product of 238 bp. C.) For comparison, a 6-plex reaction containing 12 product strands is shown. D.) For a contrasting comparison, the raw spectrum charge state distribution is shown for a single double-stranded product for locus DYS392 where the two strands are less than half the size of those shown in panel A.

allows 24 samples to be run on each assay plate (or 22 samples plus a positive and negative control sample). This layout covers 15 Y-STR loci and consists of one 6-plex reaction, one 7-plex reaction, and two single-plex reactions. In addition, an optional layout would allow 16 loci (including DYS448) in five reactions per sample. The product size range for DYS448 with our currently selected primer pair (primer pair 4902 from Table 27) is 202 to 244 base pairs, which results in too many charge states in the raw mass spectrum to multiplex this primer pair with other primer pairs (see Figure 28). Inclusion of DYS448 in a final assay would therefore require three single-plex reactions (DYS448, DYS19 and DYS385a/b), reducing the number of samples that could be run on a single 96-well assay plate to 12 (see Figure 29). Two layout options that have been tested are shown in Figure 29. Data for a blood-derived sample (55-24622) is shown in Figure 30 for layout option

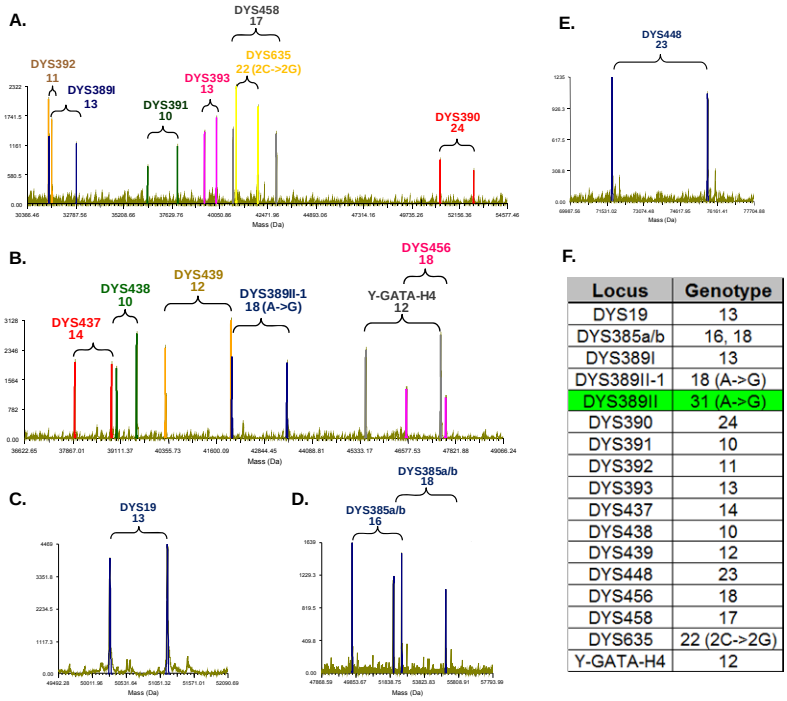
A.

Reaction	pp num	Locus	Primer sequences
Reaction 1 (7-plex)	4586	DYS389I	TCCAACTCTCATCTGTATTATCTATGTATCTG TAGATAGATTGATAGAGGGAGGGATAGATAG
	4597	DYS392	TGGAAAACAAATTTTTTCCTTGTATCACCA TCCATTAAACCTACCAATCCCATTCC
	4594	DYS391	TCATTCAATCATACACCCATATCTGTCTG TGCAAGCAATTGCCATAGAGG
	4601	DYS393	TGGTGGTCTTCTACTTGTGTCAATAC TGGAACTCAAGTCCAAAAAATGAGG
	4591	DYS390	TCTGCATTTTGGTACCCCATAATATTTC TGTGTATACTCAGAAACAAGGAAAGATAGATA
	4924	DYS458	TCTGAGCAACAGGAATGAAACTC TGAGCCACCACGCCCAC
	4910	DYS635	TCCCCGGATAAAGAAAATGTGATAGATAGATAG TGGCTTCTCACTTTGCATAGAATCTCTATC
Reaction 2 (6-plex)	4587	DYS389II-1	TCCAACTCTCATCTGTATTATCTATGTGTG TGATGAGAGTTGGATACAGAAGTAGGTATAATG
	4611	DYS438	TCCAAAATTAGTGGGGAATAGTTGAACG TAGCCTGGGCAACAAGAGTG
	4615	DYS439	TAGATAGATACATAGGTGGAGACAGATAGATG TGCTTGGAATTCTTTTACCCATCATCTC
	4608	DYS437	TCGTGAGTGCATGCCCATC TCGTTCATAGATAAGTAGATAGACATCATTCAC
	4929	DYS456	TGGGACCTTGTGATAATGTAAGATAGA TGCCCAAAACTTCTTAAACTGATGTATTAG
	4912	Y-GATA-H4	TCCGCTGAGGAGAATTTCCAAATTTAAG TCCCCTATCTATCTATTCATCCATCTAATCTATCC
Reaction 3	4579	DYS19	TGCCTACTGAGTTTCTGTTATAGTGTTTTTT TCATCTGGGTTAAGGAGAGTGTCAC
Reaction 4	4692	DYS385a/b	TTTAAAGAGAAAGAGGAAAGAGAAAGAAAGG TGTGGGATAATCTATCTATTCCAATTACATAGTC
Reaction 5	4902	DYS448	TGAGACAGAAAGGGAGATAGAGACATGG TATTTCTGGCCGGTCTGGAAATTTATCTC

B.

Reaction	pp num	Locus	Primer sequences
Reaction 1 (5-plex)	4591	DYS390	TCTGCATTTTGGTACCCCATAATATATTC TGTGTATACTCAGAAACAAGGAAAGATAGATA
	4594	DYS391	TCATTCAATCATACACCCATATCTGTCTG TGCAAGCAATTGCCATAGAGG
	4597	DYS392	TGGAAAACAAATTTTTTCCTTGTATCACCA TCCATTAAACCTACCAATCCCATTCC
	4611	DYS438	TCCAAAATTAGTGGGGAATAGTTGAACG TAGCCTGGGCAACAAGAGTG
	4615	DYS439	TAGATAGATACATAGGTGGAGACAGATAGATG TGCTTGGAATTCTTTTACCCATCATCTC
Reaction 2 (5-plex)	4587	DYS389II-1	TCCAACTCTCATCTGTATTATCTATGTGTG TGATGAGAGTTGGATACAGAAGTAGGTATAATG
	4601	DYS393	TGGTGGTCTTCTACTTGTGTCAATAC TGGAACTCAAGTCCAAAAAATGAGG
	4608	DYS437	TCGTGAGTGCATGCCCATC TCGTTCATAGATAAGTAGATAGACATCATTCAC
	5157	DYS456	TGTGGGACCTTGTGATAATGTAAGATAGATAG TTCTCTAGAGGGACAGAACTAATGGAATATC
	4912	Y-GATA-H4	TCCGCTGAGGAGAATTTCCAAATTTAAG TCCCCTATCTATCTATTCATCCATCTAATCTATCC
Reaction 3 (4-plex)	4579	DYS19	TGCCTACTGAGTTTCTGTTATAGTGTTTTTT TCATCTGGGTTAAGGAGAGTGTCAC
	4586	DYS389I	TCCAACTCTCATCTGTATTATCTATGTATCTG TAGATAGATTGATAGAGGGAGGGATAGATAG
	4924	DYS458	TCTGAGCAACAGGAATGAAACTC TGAGCCACCACGCCCAC
	4910	DYS635	TCCCCGGATAAAGAAAATGTGATAGATAGATAG TGGCTTCTCACTTTGCATAGAATCTCTATC
Reaction 4	4692	DYS385a/b	TTTAAAGAGAAAGAGGAAAGAGAAAGAAAGG TGTGGGATAATCTATCTATTCCAATTACATAGTC
Reaction 5	4902	DYS448	TGAGACAGAAAGGGAGATAGAGACATGG TATTTCTGGCCGGTCTGGAAATTTATCTC

Figure 29. Five-reaction layouts containing the 16 Y-STR loci used in the Y-Filer™ system. A. Primer pairs and reaction groupings for layout option 1. B. Primer pairs and reaction groupings for layout option 2.



Locus	Genotype
DYS19	13
DYS385a/b	16, 18
DYS389I	13
DYS389II-1	18 (A->G)
DYS389II	31 (A->G)
DYS390	24
DYS391	10
DYS392	11
DYS393	13
DYS437	14
DYS438	10
DYS439	12
DYS448	23
DYS456	18
DYS458	17
DYS635	22 (2C->2G)
Y-GATA-H4	12

Figure 30. Sixteen-locus Y-STR assay performed in five reaction wells. A.) Reaction 1 consists of a seven-plex reaction containing loci DYS389I, DSY390, DYS391, DYS392, DYS393, DYS458 and DYS635. B.) Reaction 2 consists of a 6-plex reaction containing loci DYS389II-1 (half of DYS389, specifically amplified independently of DYS389I), DYS437, DYS438, DYS439, DYS456 and Y-GATA-4H. C.), D.) and E.) Reactions 3, 4 and 5 are each single-plex reactions containing loci DYS19, DYS385a/b and DYS448, respectively. F.) 16-locus profile for the sample for which data are shown in panels A-E. Note that the genotype for DYS389II, for backwards compatibility with existing assays and databases, can be obtained by simply adding the base allele designations for DYS389I and DYS389II-1 (highlighted in green in panel F).

1. Note that one primer pair (the primer pair for DYS456) from layout 1 was modified for layout 2 and the 8-well layout presented below due to potential cross-reactivity with high concentrations of X-chromosome (>3 ng female DNA in the absence of male DNA, or ≥25-fold excess of female DNA over male DNA – see Species Specificity section below). Also, at high levels of female DNA, the primer pairs for DYS439 and Y-GATA-H4 created cross products with female DNA when in combination in the same reaction, but not when separated, so these two products were put in different multiplexes.

Although difficult to see in the deconvolved data view in Figure 28, the current baseline noise in the seven-plex reaction (Figure 30, panel A) is higher than desirable. We have explored working reducing the noise baseline and improving product balance

A.

Primer Pair Number	Locus	Primer sequences
4579	DYS19	TGCCTACTGAGTTTCTGTTATAGTGTTTTTT TCATCTGGGTTAAGGAGAGTGTCAC
4692	DYS385a/b	TTTAAAGAGAAAGAGGAAAGAGAAAGAAAGG TGTGGGATAATCTATCTATTCCAATTACATAGTC
4586	DYS389I	TCCAACTCTCATCTGTATTATCTATGTATCTG TAGATAGATTGATAGAGGGAGGGATAGATAG
4587	DYS389II-1	TCCAACTCTCATCTGTATTATCTATGTGTG TGATGAGAGTTGGATACAGAAGTAGGTATAATG
4591	DYS390	TCTGCATTTTGGTACCCCATAATATATTC TGTGTATACTCAGAAACAAGGAAAGATAGATA
4594	DYS391	TCATTCAATCATACACCCATATCTGTCTG TGCAAGCAATTGCCATAGAGG
4597	DYS392	TGGAAAACAAATTTTTTCCTTGTATCACCA TCCATTAAACCTACCAATCCCATTCC
4601	DYS393	TGGTGGTCTTCTACTTGTGTCAATAC TGGAACTCAAGTCCAAAAATGAGG
4608	DYS437	TCGTGAGTGCATGCCCATC TCGTTCATAGATAAGTAGATAGACATCATTCAC
4611	DYS438	TCCAAAATTAGTGGGGAATAGTTGAACG TAGCCTGGGCAACAAGAGTG
4615	DYS439	TAGATAGATACATAGGTGGAGACAGATAGATG TGCTTGGAATTCTTTTACCCATCATCTC
4902	DYS448	TGAGACAGAAAGGGAGATAGAGACATGG TATTTCTGGCCGGTCTGGAAATTTATCTC
5157	DYS456	TGTGGGACCTTGTGATAATGTAAGATAGATAG TTCTCTAGAGGGACAGAACTAATGGAATATC
4924	DYS458	TCTGAGCAACAGGAATGAAACTC TGAGCCACCACGCCCAC
5139	DYS635	TCCCCGGATAAAGAAAATGTGATAGATAGATAG TCTTGGCTTCTCAGTTTGCATAGAATC
4912	Y-GATA-H4	TCCGCTGAGGAGAATTTCCAAATTTAAG TCCCCTATCTATCTATTCATCCATCTAATCTATCC

B.

Reaction 1		
pp	locus	conc
4692	DYS385a/b	500

Reaction 2		
pp	locus	conc
4902	DYS448	500

Reaction 3		
pp	locus	conc
4591	DYS390	250

Reaction 4		
pp	locus	conc
4587	DYS389II-1	315
4601	DYS393	200

Reaction 5		
pp	locus	conc
4611	DYS438	500
5139	DYS635	300

Reaction 6		
pp	locus	conc
4586	DYS389I	150
4594	DYS391	225
4924	DYS458	400

Reaction 7		
pp	locus	conc
4579	DYS19	400
4608	DYS437	100
4912	Y-GATA-H4	200

Reaction 8		
pp	locus	conc
4597	DYS392	150
4615	DYS439	300
5157	DYS456	330

C.

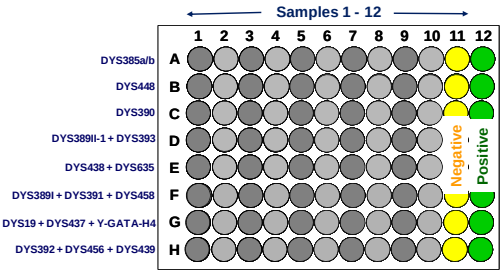


Figure 31. Primer pairs, concentrations and assay layout for 8-well Y-STR assay covering 16 loci. A.) The primer pairs for each of the 16 loci covered in the current assay. B.) Primer pair reaction combinations and concentrations used in final reactions (in nM). C.) Intended assay layout on a 96-well plate.

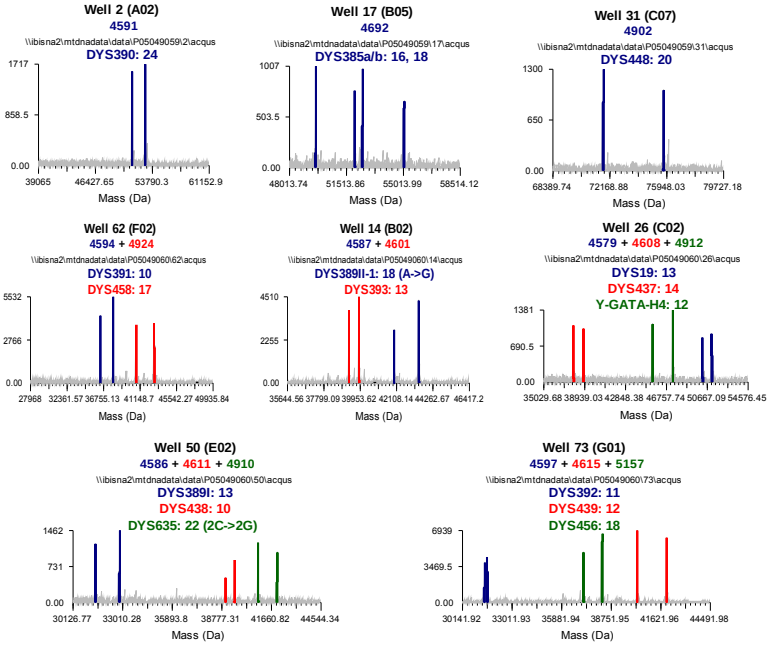


Figure 32. Deconvolved spectral outputs from 1 ng/reaction of current 8-well Y-STR reaction layout (Figure 31) using representative blood sample 55-24622.

by experimenting with thermocycling programs, primer pair concentrations and iterative removal of different primer pairs to try to identify primer pairs that may need to be redesigned. The best results have so far been achieved with a rearranged layout utilizing eight reactions per sample to minimize noise baselines and potential cross-reactivity with female DNA. The eight-well assay layout is configured as shown in Figure 31. Profile data produced with 1 ng/reaction of representative blood sample 55-26422 is shown in Figure 32.

Final reaction formulations consist of 20 mM Tris-Cl, 75 mM KCl, 1.5 mM $MgCl_2$, 400 mM betaine, 20 mM sorbitol, 200 μM each dATP, dCTP, dTTP, 200 μM ^{13}C-enriched dGTP (obtained from Cambridge Isotope Laboratories, Andover, MA – between 99 and 99.6% enriched as determined by internal monitoring at Ibis), 5 U AB AmpliTaq Gold (Life Technologies, Carlsbad, CA), and primer pair concentrations shown in Figure 55. Reactions are formulated at a 1.143X concentration and 35 μL of each reaction formulation is placed in the appropriate wells of a 96-well plate according to the layouts shown in Figures 31 or 29. Pre-fabricated PCR plates are heat-sealed and frozen at -20 °C prior to use in thermocycling. At this time, stability testing has not been performed with the Y-STR kit plate. Thermocycling parameters consist of [$96^{\circ}_{10\ min}$, [$96^{\circ}_{20\ sec}$, $58^{\circ}_{1.5\ min}$, $72^{\circ}_{45\ sec}$]$_{40\ cycles}$, $72^{\circ}_{4\ min}$, 4 $^{\circ}C_{hold}$], using a 5% ramp rate for the melt-anneal transition (~0.225°C/sec). Plate setup consists of pipetting 5 μL of purified DNA template into the 8 wells of one column on the assay plate for each sample, resealing the plate, and thermocycling. After thermocycling, the plate is set directly on the T5000 or PLEX-ID instrument and all downstream steps up to final analysis and data QC are automated.

2.2. Sensitivity

4-well, 13 locus assay

The Y-STR assay configuration shown in Table 29 was tested in duplicate 2-fold dilution series with template inputs from 1 ng/reaction to 7.8 pg/reaction. Human blood-derived DNA concentrations were determined using the Quantifiler assay. Of two blood-derived DNA templates tested, one produced a full profile with both duplicates at 62.5 pg/reaction (Figure 33). The other produced a full profile with one replicate at 62.5 pg/reaction and one replicate at 125 pg/reaction, but had locus drop-outs in the other replicate at both concentrations.

A. Blood sample KTMAM-C

Template Quantity (pg)	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS456	DYS458
1000	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
500	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
250	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
125	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
62.5	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
31.25	14	13, 14	12	17	23	---	11	12, 14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	12, 14	14, 16	10	11	14	16
15.6	14	13, 14	12	17	23	10	11	14	16	10	11	14	16
	14	13, 14	12	17	23	10	11	14	16	10	11	13, 14, 15	16
7.8	14	13, 14	---	16, 17	23	10	11	14	16	10	11	15	16
	14	13, 14	---	16, 17	23	10	---	14	---	10	---	14	16
Negative	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---
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	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---

B. Blood sample N31773

Template Quantity (pg)	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS456	DYS458
1000	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
500	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
250	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
125	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
	15	---	---	19 (A->G)	21	---	---	---	---	11	11	15	16
62.5	15	16, 17	13	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
	15	17, ---	---	19 (A->G)	21	10	11	13	14 (C->T)	11	11	15	16
31.25	---	---	13	19 (A->G)	21	10	11	13	14 (C->T)	11	---	15	16
	15	---	---	19 (A->G)	21	---	---	13	---	11	---	15	16
15.6	15	---	---	---	21	---	---	---	14 (C->T)	---	---	---	16
	15	---	---	---	---	10	11	---	14 (C->T)	---	---	15	---
7.8	---	---	---	---	---	---	---	---	---	---	---	---	16
	---	16, ---	---	---	20, 21	10	---	13	---	---	---	---	16
Negative	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---	---	---	---	---
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Figure 33. Sensitivity of the 4-well, 13-locus Y-STR assay. Two different templates were tested in 2-fold dilution series performed in duplicate. A. Blood sample KTMAM-C produced full profiles for both duplicates at 62.5 pg / reaction. Below 62.5 pg, drop-outs and drop-ins were observed for a few loci. No reagent contamination was apparent, as eight negative controls were run and no products were observed. B. Blood sample N31773-C produced full profiles for one duplicate at 62.5 pg / reaction and 125 pg / reaction. In one replicate at 125 pg, several loci dropped out. In one replicate at 62.5 pg/reaction, one allele dropped out from DYS385a/b, and DYS389I dropped out. Below 62.5 pg, drop-outs were observed for a few loci, and one drop-in occurred for DYS390 in one replicate at 7.8 pg. No reagent contamination was apparent, as eight negative controls were run and no products were observed.

5-well, 16 locus assay

Sensitivity studies using the primer pair layout shown in Figure 46.5 (layout option 1, utilizing a 7, plex, 6-plex and two [for 24 samples per plate] or three [for 12 samples per plate] single-plex reactions) did not perform to the level of the previous 13-locus assay shown in Figure 33. Tests using the 7-plex and 6-plex reactions indicated a lower sensitivity of 125-250 pg/reaction, largely due to the heightened noise baselines and spectral congestion observed with the addition of more primer pairs into each reaction (Figure 34).

A. Blood sample 55-24622.

Template Quantity (pg)	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
1000	13	16, 18	13	18	24	10	11	13	14	10	12	20	18	17	22	12
500	13	16, 18	13	18	24	10	11	13	14	10	12	20	18	17	22	12
250	13	16, 18	13	18	24	no data	no data	13	14	10	12	20	18	17	22	12
125	13	16, 18	13	18	24	10	11	13	14	10	12	20	18	17	22	12
62.5	13	16, 18	13	18	24	10	no data	13	14	10	12	20	18	17	22	12
31.25	13	16, 18	13	18	24	no data	no data	13	14	no data	12	20	no data	no data	22	12
15.6	13	18, 18	no data	18	24	no data	11	13	no data	no data	12	20	18	17	no data	12
7.8	13	18, 18	no data	18	no data	no data	11	no data	14	10	12	no data	no data	17	no data	no data
3.9	13	no data	no data	no data	24	no data	11	no data	no data	no data	no data	no data	14, 18	no data	no data	no data
1.95	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	12
0.98	13	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data
Negative	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data

B. Blood sample LB.

Template Quantity (pg)	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
1000	14	13, 15	12	17	23	11	11	12	14	10 (A->C)	11	21	15	16.2	21 (2C->2G)	11
500	14	13, 15	12	17	23	11	11	12	14	10 (A->C)	11	21	15	16.2	21 (2C->2G)	11
250	14	13, 15	12	17	23	11	11	12	14	10 (A->C)	11	21	15	16.2	21 (2C->2G)	11
125	14	13, 15	12	17	23	11	11	12	14	10 (A->C)	11	21	15	16.2	21 (2C->2G)	11
62.5	14	13, 15	no data	17	23	no data	no data	no data	14	10 (A->C)	11	21	15	16.2	no data	11
31.25	14	13, 15	no data	17	no data	no data	no data	12	14	no data	11	21	15	no data	no data	11
15.6	14	15, ---	no data	17	no data	no data	no data	12	14	no data	11	no data	15	16.2	no data	no data
7.8	no data	no data	no data	no data	no data	no data	no data	no data	14	10 (A->C)	no data	no data	no data	no data	no data	no data
3.9	14	no data	no data	no data	no data	no data	no data	no data	14	no data	no data	no data	no data	no data	no data	no data
1.95	no data	13, ---	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	16.2	no data	no data
0.98	no data	no data	no data	16	no data	no data	no data	no data	no data	no data	11	no data	no data	no data	no data	11
Negative	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data	no data

Figure 34. Sensitivity of 16-locus assay configured in the 5-well layout shown in Figure 46.5. Using two different male templates, full profiles were detected at 125 pg/reaction, but not below that level.

8-well, 16 locus assay

The 8-well assay layout shown in Figure 31 was in part an effort to achieve the same sensitivity seen with 11 and 13-locus preliminary assays and to eliminate cross-reactivity with the X-chromosome (see Species specificity section below). Dilution series from 1000 pg per reaction to 1.0 pg per reaction were performed in duplicate on six independent DNA templates that had been purified from blood (using Gentra Puregene Blood Kit (Qiagen, Valencia, CA), and then

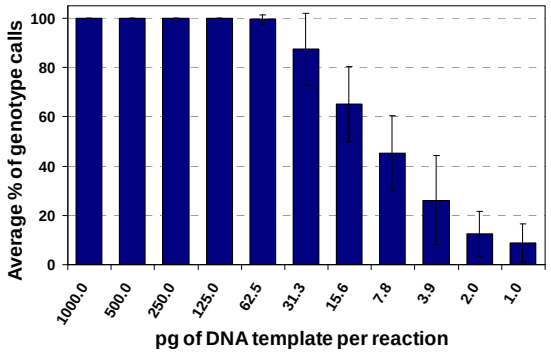


Figure 35. Sensitivity of 8-well Y-STR assay using purified DNA template. Sensitivity was measured with duplicate dilution series of six different templates prepared from male human blood. The average percentage of loci correctly called (out of 16) for each of the 12 dilution series is graphed ± the standard deviation for the 12 runs. At 62.5 pg per reaction, one allele was missed from DYS437 (allele 15) from one replicate of sample 55-25290.

polished with organic extraction and precipitation). DNA samples were quantified using the AB Quantifiler™ assay (Life Technologies, Carlsbad, CA) as well as A_{260} using a NanoDrop UV spectrophotometer (Thermo Scientific NanoDrop products, Wilmington, DE). Full profiles were detected in 11 of 12 replicates at 62.5 pg/reaction (one allele call was missed in one replicate). Full profiles were produced at 125, 250, 500 and 1000 pg/reaction (Figure 35).

2.3. Species specificity

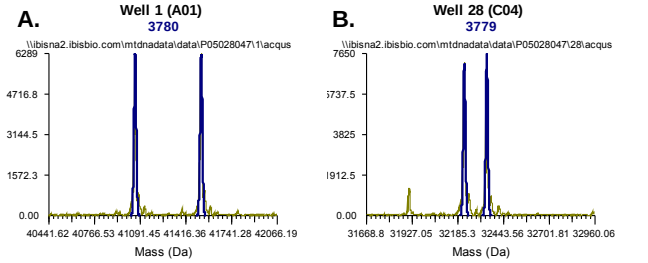


Figure 36. Confirmation of amplifiable dog and cat DNA. 1 ng canine DNA (A.) or feline DNA (B.) in the presence of primer pairs specific to the canine Y-chromosomal Sry gene (A.) or the feline Y-chromosomal Sry gene (B). A single product with the expected base composition [A43 G36 C30 T23] for canine (A.) or [A29 G29 C29 T16] for feline was produced (B.).

Male DNA has been tested in the presence of a 10-fold excess of DNA from six different non-human species at three stages of assay development. DNA from two vertebrate species (domestic dog and cat), filamentous fungus (*Aspergillus oryzae*), yeast (*Candida albicans*), gram negative bacteria (*Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus*) was used at 10 ng per reaction in the absence and presence of 1 ng of human DNA in the 4-well, 13-locus assay, (data not shown, but reported in earlier progress reports), the 5-well, 16-locus assay and the 8-well, 16-locus assay. DNA concentrations of exogenous DNA templates were determined spectrophotometrically by absorbance at 260 nm. The bacterial and fungal DNAs have been used multiple times as controls in bacterial and fungal detection assays and have been multiply confirmed to contain amplifiable DNA. The dog and cat DNAs were confirmed as amplifiable and as male by testing them with primer pairs made internally and directed at the SRY element[48] (Figure 36).

For the 5-well, 16-locus assay, DNA from in-house blood sample 55-24622 was used at 1 ng/reaction in the presence of a 10-fold excess (10 ng/reaction) of the above-mentioned six exogenous DNAs. All reactions were run in triplicate. Data for a single representative test in the presence of dog DNA are shown in Figure 37. For each of the six exogenous DNAs tested, triplicate control tests were also run with 10 ng of exogenous DNA with no human DNA added to test for amplified products. No specific products were detected in these tests. Figure 37 shows an example of each reaction in the presence of 10 ng of dog DNA without human DNA below each spectrum showing the reaction with human DNA. For all assays performed with human DNA in the presence of a 10-fold excess of exogenous DNA, a full profile was produced, with the exception of four isolated reaction failures yielding no results, and no interference from the exogenous DNAs was apparent (not shown). Each of the four reaction failures was

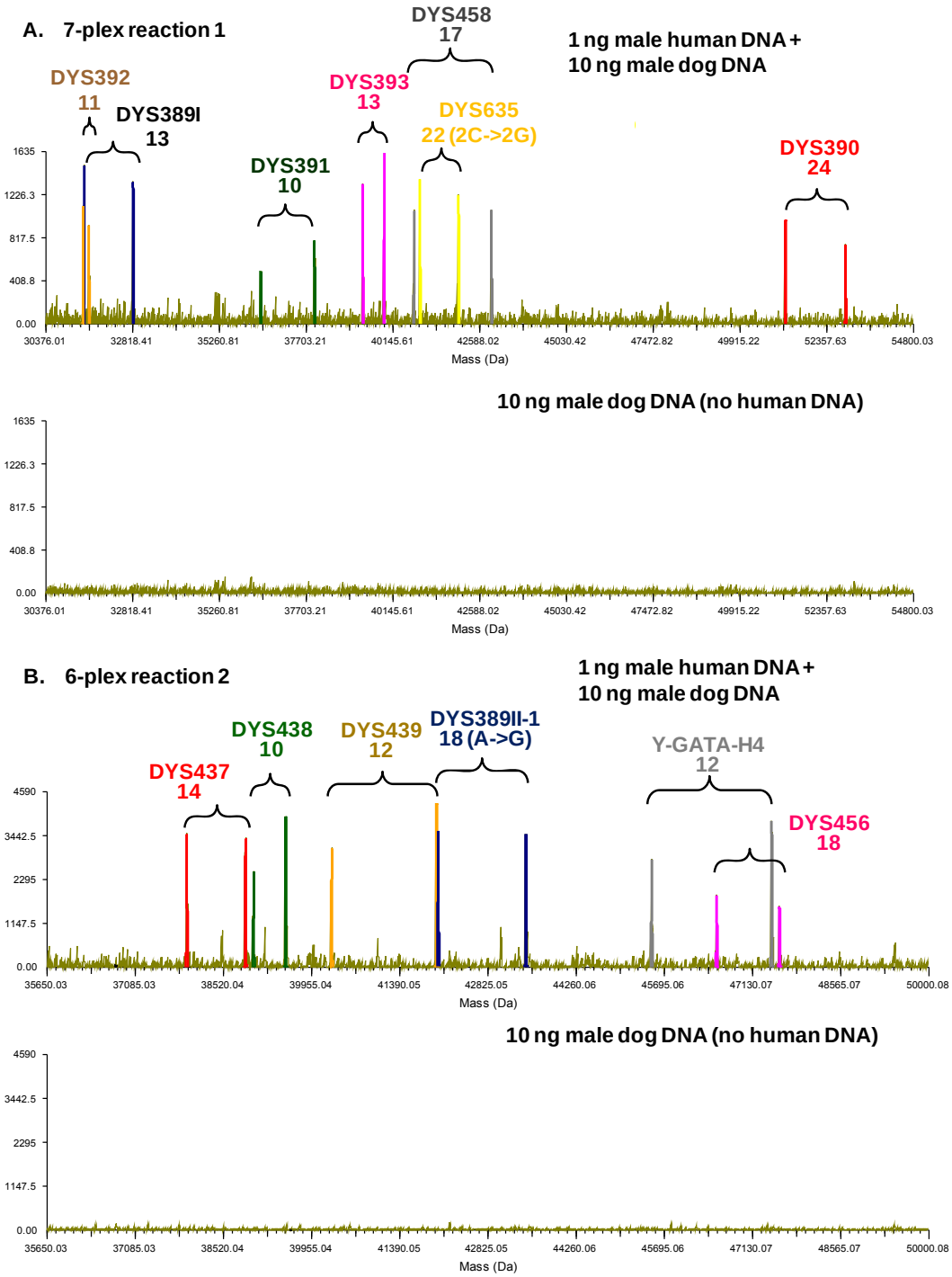


Figure 37. Species specificity: 1 ng of human DNA run in the presence of 10 ng of dog DNA. A single representative reaction for 1 ng of human DNA run in the presence of 10 ng of dog DNA is shown for each reaction in the assay layout. All reactions were run in triplicate. A.) 7-plex reaction 1. B.) 6-plex reaction 2.

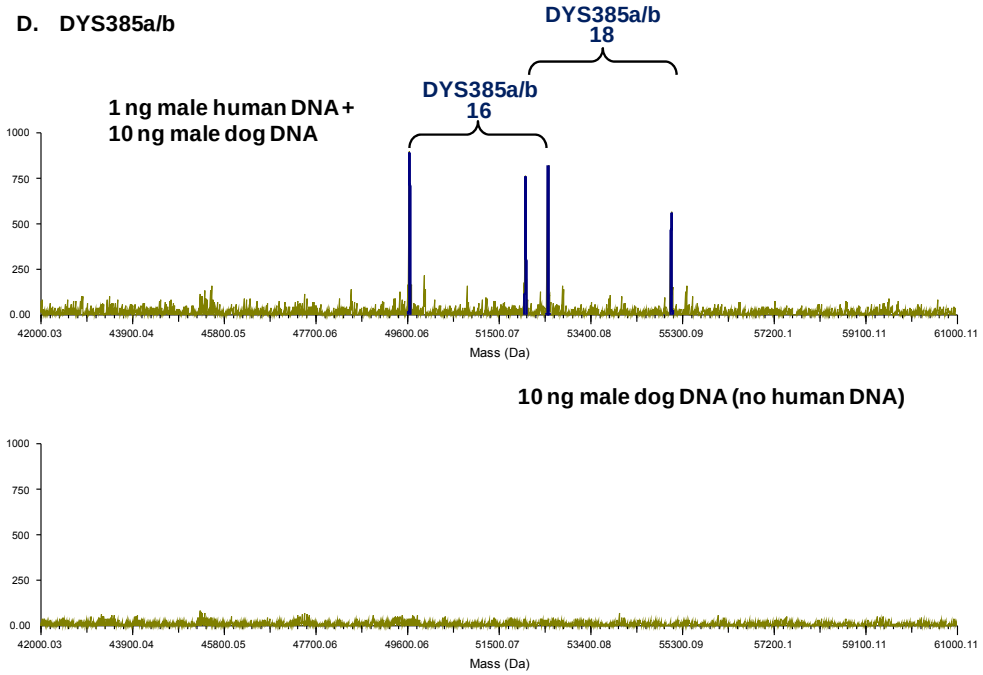


Figure 37, continued. Species specificity: 1 ng of human DNA run in the presence of 10 ng of dog DNA. A single representative reaction for 1 ng of human DNA run in the presence of 10 ng of dog DNA is shown for each reaction in the assay layout. All reactions were run in triplicate. C.) Single-plex reaction for DYS19. D.) Single-plex reaction for DYS385a/b.

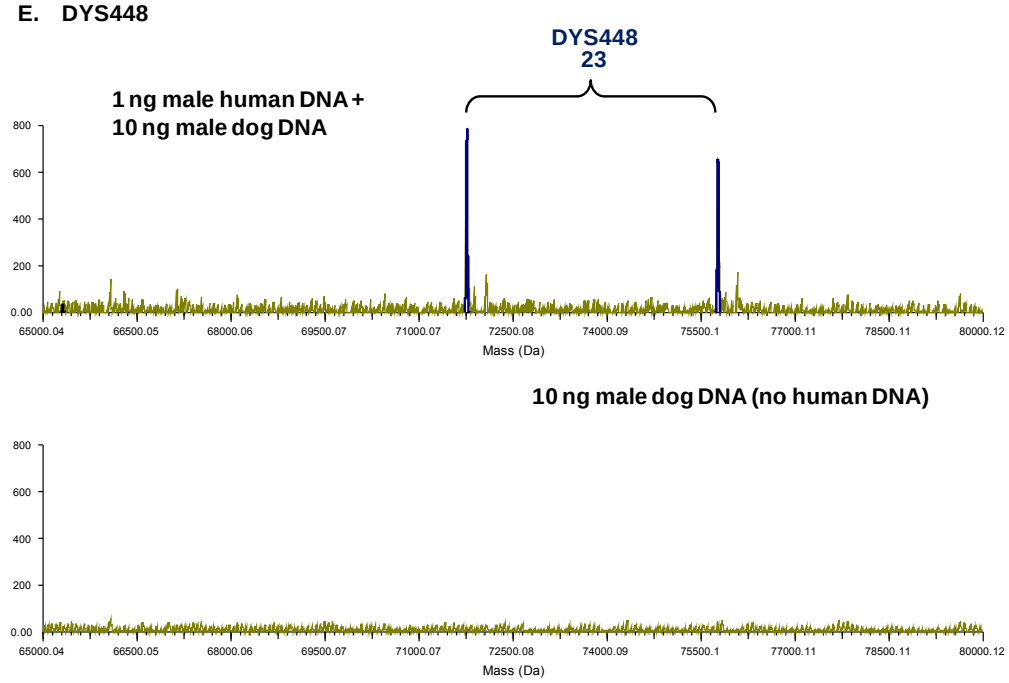


Figure 37, continued. Species specificity: 1 ng of human DNA run in the presence of 10 ng of dog DNA. A single representative reaction for 1 ng of human DNA run in the presence of 10 ng of dog DNA is shown for each reaction in the assay layout. All reactions were run in triplicate. E.) Single-plex reaction for DYS448.

an isolated case of one of three replicates where the other two reactions worked normally and each appeared to be related to reaction setup.

Species specificity experiments were repeated with the final 8-well assay layout with the same exogenous DNAs in triplicates. Spectra generated from reactions containing exogenous DNA with and without human DNA looked qualitatively similar to those seen with the 5-well assay layout (Figure 37, data not shown redundantly). Reactions containing 1 ng of human DNA with 10 ng of exogenous DNA produced spectra that were qualitatively identical to those containing 1 ng human DNA with no exogenous DNA background, and no specific products were detected at any appreciable level from exogenous templates alone (not shown). All reactions containing human DNA produced full profiles and reactions without human DNA did not produce products (Table 29).

Table 29. Species specificity. Y-STR profiles determined for human DNA (blood sample 55-24622) in the presence of six different exogenous DNAs present at a 10-fold excess (by mass) over the human DNA. 10 ng per reaction of exogenous DNA was run in all reactions except the six replicates labeled with "None", in which case dilution buffer was used as a negative control.

Human Template	Exogenous Template	Rep	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
1 ng	Dog	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Cat	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Aspergillus oryzae	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Candida albicans	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Escherichia coli	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Stphylococcus aureus	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	None	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
		3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
none	Dog	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Cat	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Aspergillus oryzae	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Candida albicans	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Escherichia coli	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Stphylococcus aureus	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	None	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
		3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

In addition to the above-mentioned species, four male primate blood samples were obtained from BioMed Supply Inc. (Carlsbad, CA) and DNA was purified (GenePure kit, Qiagen, Valencia, CA) and quantified by UV absorbance at 260 nM. The 8-well assay layout was tested with 10 ng per reaction of these DNAs in the absence and the presence of 1 ng of male human DNA. Species tested were marmoset monkey, squirrel monkey, African green monkey, and rhesus monkey. All reactions were run in triplicate. Specific products were not detected from the four primate species tested. In one of the triplicate experiments for African green monkey, a single low-level detection of an allele 22 (2C->2G) was detected for DYS635 that was not detected in the replicates (not

shown). The triplicate run was repeated and the allele was not detected in any of the three replicates. The presence of a 10-fold excess of these four DNAs did not appear to interfere with correct profile detection (Table 30).

Table 30. Species specificity. Y-STR profiles determined for human DNA (blood sample 55-24622) in the presence of four different exogenous primate DNAs present at a 10-fold excess (by mass) over the human DNA. 10 ng per reaction of exogenous DNA was run in all reactions except the six replicates labeled with "None", in which case dilution buffer was used as a negative control.

Human Template	Exogenous Template	Rep	DYS19	DYS385a/b	DYS389I	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
1 ng	African green monkey	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	African green monkey	2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	African green monkey	3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Marmoset monkey	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Marmoset monkey	2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Marmoset monkey	3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Rhesus monkey	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Rhesus monkey	2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Rhesus monkey	3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Squirrel monkey	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Squirrel monkey	2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	Squirrel monkey	3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	None	1	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	None	2	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
	None	3	13	16, 18	13	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
none	African green monkey	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	African green monkey	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	African green monkey	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Marmoset monkey	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Marmoset monkey	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Marmoset monkey	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Rhesus monkey	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Rhesus monkey	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Rhesus monkey	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Squirrel monkey	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Squirrel monkey	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	Squirrel monkey	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	None	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	None	2	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	None	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Specificity to male DNA:

To test the primer pairs and assay for specificity to male human DNA, the preliminary 5-well assay was first tested in the presence of up to 100 ng of human female DNA. Female DNA (in-house blood sample 55-24781) was quantified with both Quantfiler™ and spectrophotometrically by UV absorbance at 260 nm. DNA from female sample 55-24781 was tested in the Y-STR assay at template input concentrations of 100 ng/reaction to 0.8 ng/reaction in 2-fold serial dilutions. Female DNA was tested alone for the generation of products in the absence of specific male template and in the presence of 1 ng/reaction male DNA for the possibility of interference with correct genotyping of male DNA.

In the absence of male DNA, the 6-plex reaction (reaction 2) produced three distinct products at 100 ng of input DNA (see Figure 38, panel B). These products were not mistaken for male Y-chromosomal markers and it was not immediately evident which primer pair(s) produce them, or if they are generated by a mix-matching of primers between multiplexed pairs. The products were visible in reaction 2 down to about 3 ng of input female DNA template in the absence of any male DNA to compete with them (not shown). However, even at 100 ng of female in the presence of 1 ng male DNA, the male DNA effectively competed for the PCR reactions and normal profiles were produced for all loci (Figure 38). No other amplified products were observed with female DNA whether run by itself or in the presence of male DNA. Full profiles were produced for the male DNA run at 1 ng in the presence of all

concentrations of female DNA. In the presence of male DNA, the female-specific products in reaction 2 were visible down to about 25 ng rather than 3 ng (not shown).

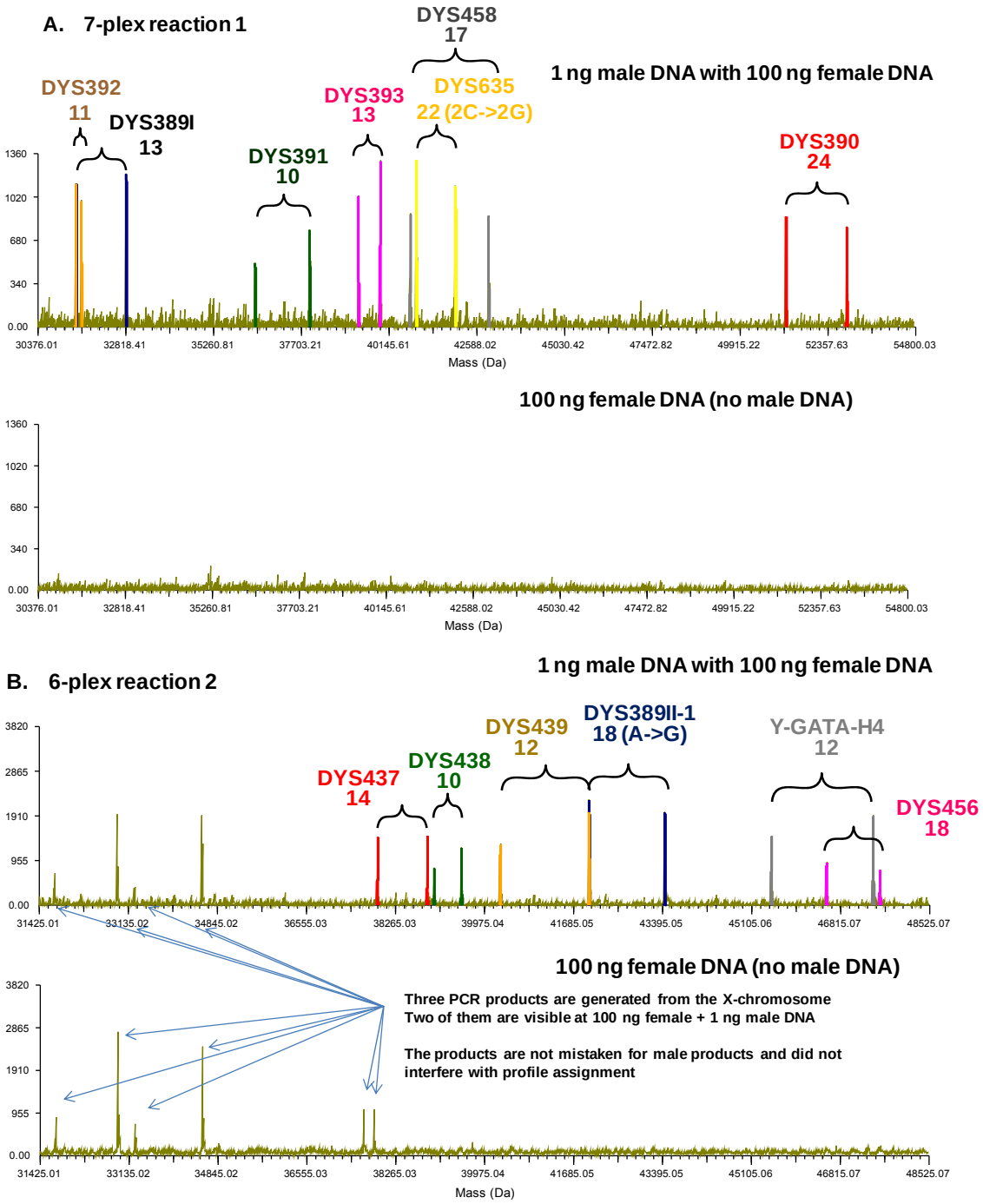


Figure 38. Specificity to human male DNA: 1 ng of male DNA run in the presence of 100 ng of human female DNA. A single representative reaction for 1 ng of human male DNA run in the presence of 100 ng of human female DNA is shown for each reaction in the assay layout. A.) 7-plex reaction 1. B.) 6-plex reaction 2.

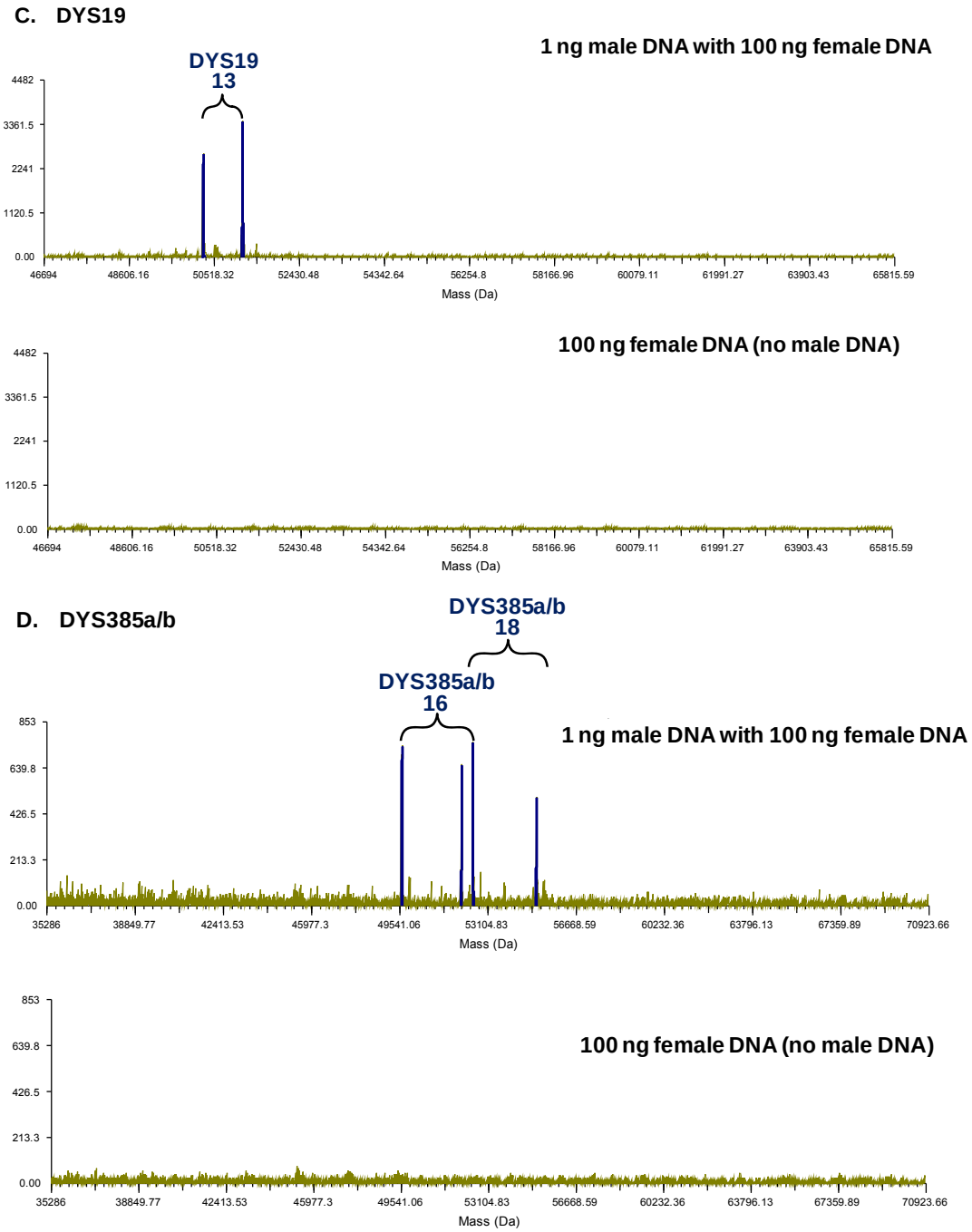


Figure 38. Specificity to human male DNA: 1 ng of male DNA run in the presence of 100 ng of human female DNA. A single representative reaction for 1 ng of human male DNA run in the presence of 100 ng of human female DNA is shown for each reaction in the assay layout. C.) Single-plex reaction for DYS19. D.) Single-plex reaction for DYS385a/b.

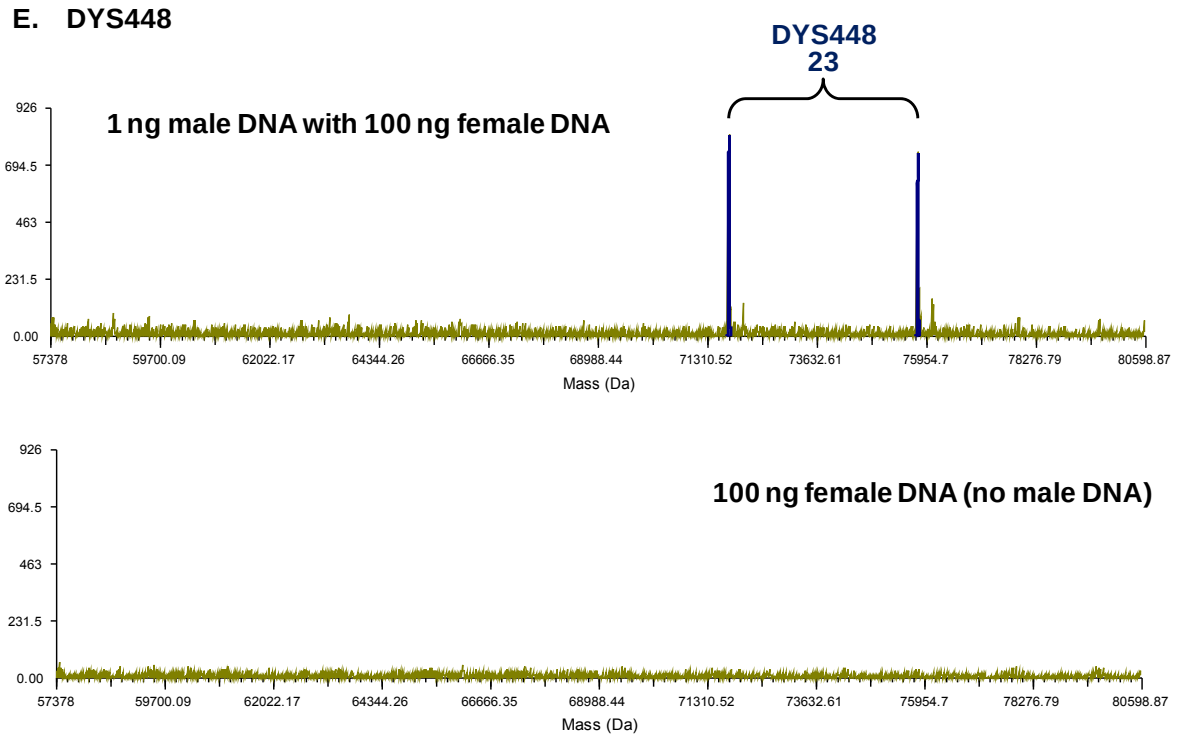


Figure 38. Specificity to human male DNA: 1 ng of male DNA run in the presence of 100 ng of human female DNA. A single representative reaction for 1 ng of human male DNA run in the presence of 100 ng of human female DNA is shown for each reaction in the assay layout. E.) Single-plex reaction for DYS448.

In order to begin to identify the source of the PCR products observed in the 6-plex in the presence of female DNA, primer pairs were individually tested with female DNA from female blood-derived sample 55-24781 at 100 ng/reaction. Primer Pair 4929 generated up to five PCR products from the X-chromosome, which were not mistaken for Y-chromosome derived products (Figure 39). To investigate the possible source of the products generated from the X-chromosome with the DYS456 primer pair 4929, BLAST searches against the published human genome were performed using the DYS456 locus from the Y-chromosome (GenBank sequence accession # AC010106.2). Potential paralogous subsequences of the X-chromosome were returned in the BLAST output that suggest a mechanism for the cross-reactivity observed with primer pair 4929. Using the apparent paralogous subsequence from X-chromosome submission GenBank accession # NT_011651.17,

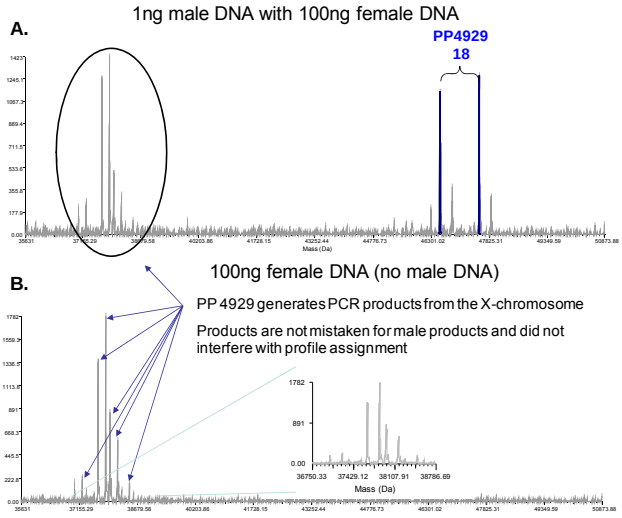
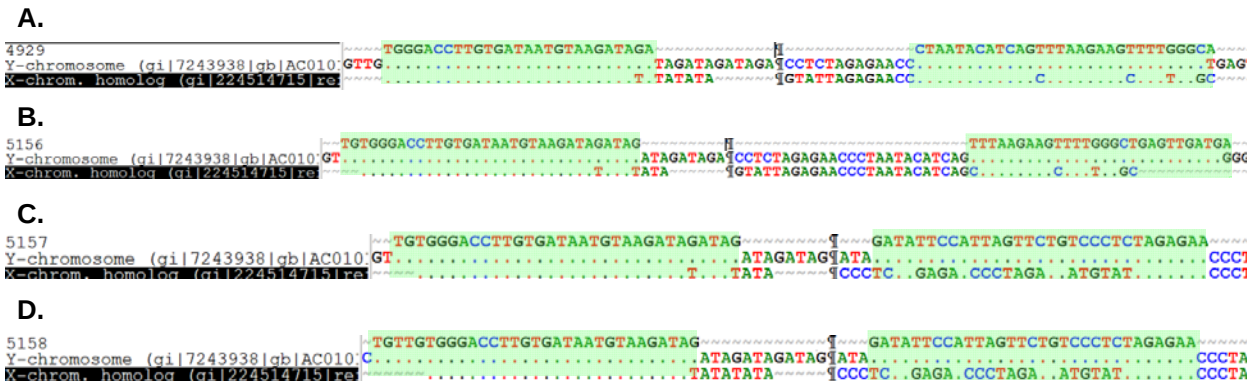


Figure 39. X-chromosomal cross-reactive PCR products produced by primer pair 4929.



E.

Ibis primer pair number	Primer sequences
5156	TGTGGGACCTTGTGATAATGTAAGATAGATAG TCATCAACTCAGCCCAAAACTTCTTAAA
5157	TGTGGGACCTTGTGATAATGTAAGATAGATAG TTCTCTAGAGGGACAGAACTAATGGAATATC
5158	TGTTGTGGGACCTTGTGATAATGTAAGATAG TTCTCTAGAGGGACAGAACTAATGGAATATC

Figure 40. Alternative primer pair selections for DYS456 to exclude paralogous sequence matches on the X-chromosome. A. The original primer pair 4929, which produced up to 5 non-Y-chromosomal products in the presence of isolated female DNA without producing Y-chromosomal products. In the presence of male DNA, the correct DYS456 product is produced, and a lower amount of the apparent paralogous cross-products are produced. There is a plausible primer-target match for both primers. The forward primer has a mismatch to the paralog one base from the 3′ end, suggesting the mechanism by which the Y-chromosomal target generally outcompetes the X-chromomsome cross-product in male DNA, and even the 1:201 ratio of X:Y target encountered in a 1:100 ratio of male:female DNA. B-D. Alternative primer pairs 5156, 5157 and 5158 have both forward and reverse primers positioned such that the 3′ terminal base of each primer does not match the paralogous template on the X-chromosome. Primer pairs 5157 and 5158 also have a largely mismatched primer relative to the X-chromosome. E. Primer pair sequences for new DYS456 primer pair candidates 5156, 5157 and 5158.

new candidate primer pairs were designed to try and eliminate the X-chromosomal cross-products (See Figure 40). Each of the new primer pairs was tested in the presence of 1 ng male DNA, 100 ng female DNA, and a mixture of 1 ng male and 100 ng female DNA. Figure 41 shows results of PCR reactions performed with male DNA (top panels) or female DNA (bottom panels), showing that the cross-reactivity to female DNA has been essentially eliminated. Reactions with male/female DNA mixtures were qualitatively the same as those with male DNA

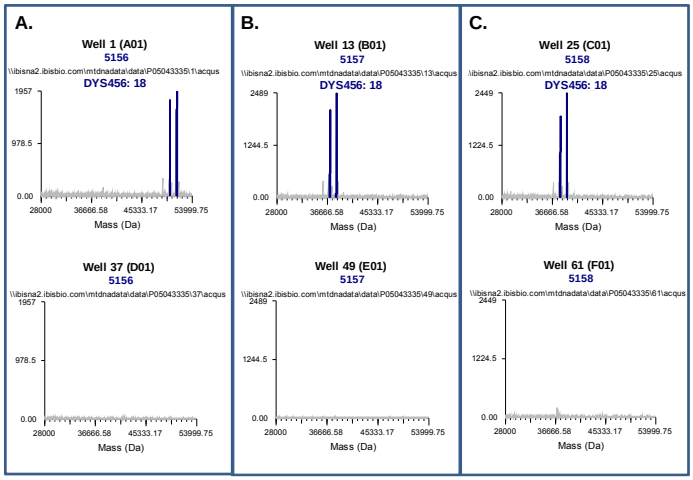


Figure 41. Mass spectra derived from PCR reactions using alternative DYS456 replacement primers 5156 (A.), 5157 (B.) and 5158 (C.). Top panels show PCR reactions using 1 ng male DNA template. Bottom panels show PCR reactions using 100 ng of female DNA. No products were produced from female DNA with the new primer pair candidates.

alone (not shown). The primer pair 5157 was chosen to move forward with based on signal output, small product size, and high male specificity of the reverse primer.

Once cross-products originating from primer pair 4929 were eliminated, multiplex testing using the replacement primer pair 5157 was resumed. In four-plex reactions containing primer pairs 4611 (DYS438,), 4615 (DYS439), 5157 (DYS456) and 4912 (Y-GATA-H4), two prominent products were observed in the presence of female DNA with masses of 32935.4/ 34563.5 Da (masses of the two strands) and 31740.9/ 33269.9 Da (Figure 42, A.). These products were not

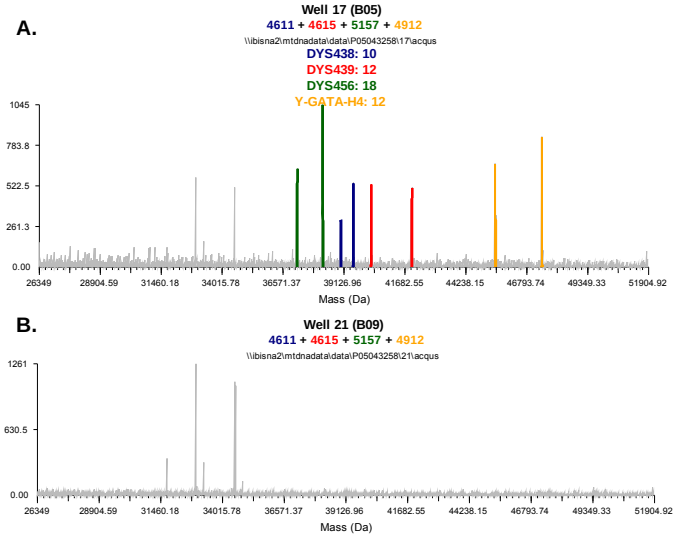


Figure 42. PCR products produced from female DNA within a multiplex reaction of primer pairs 4611, 4615, 5157 and 4912. A. 100 ng of female DNA in the presence of 1 ng male DNA. B. 100 ng of female DNA alone. Observed products are not produced by any of the primer pairs in isolation and so are presumably produced by a combination of primer from different pairs.

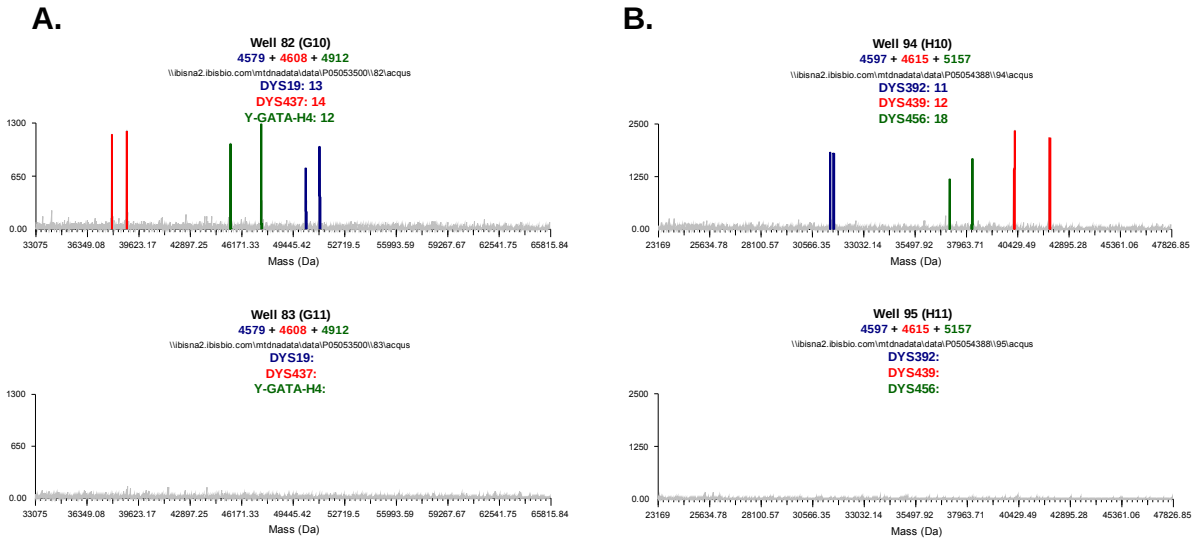


Figure 43. Elimination of prominent PCR products from female DNA within multiplexes separating primer pairs 4615 (DYS439) and 4912 (Y-GATA-H4). A. Triplex reaction containing primer pair 4912. Top panel: 1 ng of male DNA, bottom panel: 100 ng of female DNA. B. Triplex reaction containing primer pair 4615. Top panel: 1 ng of male DNA, bottom panel: 100 ng of female DNA.

produced with any of the primer pairs alone, cannot be explained by any individual primer pair's forward and reverse primers, and the more abundant product (32935.4/ 34563.5 Da) was still clearly evident in a mixture of male and female DNA (Figure 42, B.). We can deduce that the products are incompatible with any individual primer pair due to the fact that we use a ^{13}C-enriched dGTP for incorporation into PCR products. The ^{13}C-enriched dGTP is approximately 9.88 Da heavier than the natural dGTP.

Because the primers used in the PCR are not isotopically-enriched, it is possible to differentiate primer pairs compatible with production of a pair of masses when the primers in different pairs have different numbers of G's in them.

To test which primer pair combinations would produce the cross-products, all pair-wise combinations of two primer pairs were tested against female DNA. When primer pairs 4912 (Y-GATA-H4) and 4615 (DYS439) were used together in a PCR reaction, the same cross-products were produced with female DNA as were seen in the 4-plex shown in Figure 42 (data not shown). Moreover, when the forward primer from primer pair 4615 was combined with the reverse primer from 4912, the same two products are produced. An examination of potential base compositions that could be produced using the forward primer from primer pair 4615 with the reverse primer from primer pair 4912 utilizing ^{13}C-enriched dGTP in the PCR reaction suggests the base composition of [A45 G35 C4 T25] for the larger product and [A44 G33 C4 T24] for the smaller product. Note that these products differ by one A, two G's and one T, suggesting the possible amplification of a repeated region. When primer pairs 4912 and 4615 are separated in two different reactions, the cross-reactive product is not produced from female DNA (Figure 43), so multiplexes have been reconfigured to separate these two primer pairs.

2.4 Reproducibility and accuracy

Utilizing data generated with 335 runs of 214 individuals using the preliminary version of the 16-locus assay, layout version 1 from Figure 46.5 and the current 8-well assay, the distribution of mass measurement deviations from expected was assessed. The data were evaluated for 11,298 individual product strand assignments (5,649 double-stranded allele assignments). The average mass measurement deviation magnitude (absolute value of mass measurement deviations from expected) was 13.0 ± 10.8 ppm (Figure 44). 95% of mass assignments were between -35 ppm and +35 ppm relative to the expected mass for the allele. 35 ppm corresponds to a mass range of 1.0 to 2.7 Da for the range of product masses possible in the Y-STR assay (the total range of possible product masses is 30.2 kDa to 77.7 kDa). In addition, 56 runs of template 55-26422, 16 runs of template 55-25597 and 13 runs of template 55-25290 were assessed at DNA inputs ranging from 125 pg to 1 ng per reaction, producing full, correct profiles in all cases (not shown).

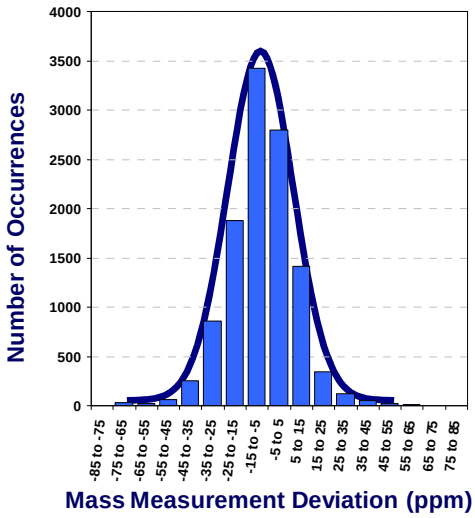


Figure 44. Distribution of mass measurement deviations for a 11,298 mass-product strand assignments produced from 335 runs of 214 male templates.

2.5 Testing against a panel of samples and population studies

Using the primer pair panel shown in Table 26, 95 male population samples[53] obtained from John Butler and Peter Vallone at NIST were tested using 1 ng/reaction of template. These samples included 31 Caucasians, 32 African Americans and 32 Hispanics. Typing results are shown in Figure 45. All 95 samples produced full profiles (there were no apparent drop-outs). Base allele calls were consistent with truth data for the 92 samples for which truth data were available (Figure 45). All 95 samples produced two alleles for locus DYS393. Unlike the control sample run for initial primer

Population	Sample	DYS19	DYS385a/b	DYS389I	DYS389II-1	Deduced* DYS389II	DYS390	DYS391	DYS392	DYS393¥	DYS437	DYS438	DYS439
African American	JT51471	15	18, 18	13	18 (A->G)	31 (A->G)	21	10	11	13, 14 (T->C)	14 (C->T)	11	12
African American	JT51499	14	13, 14	12	16	28	22	11	11	13, 14 (T->C)	16	10	11
African American	OT05888	16	16, 17	14	17 (A->G)	31 (A->G)	22	10	11	13, 14 (T->C)	14 (C->T)	11	11
African American	OT05890	15	14, 15	12	17 (A->G)	29 (A->G)	22	10	11	13, 15 (T->C)	17 (A->G)	8	12
African American	OT05892	14	14, 14	12	16	28	23	10	11	12, 14 (T->C)	16	10	11
African American	OT05893	17	16, 18	13	17 (A->G)	30 (A->G)	21	10	11	15, 15 (T->C)	14 (C->T)	11	11
African American	OT05894	15	16, 17	14	17 (A->G)	31 (A->G)	21	10	11	14, 14 (T->C)	14 (C->T)	11	11
African American	OT05896	16	16, 18	13	18 (A->G)	31 (A->G)	21	10	11	13 (T->C), 15	14 (C->T)	11	11
African American	OT05897	14	16 (G->A), 17	13	17 (A->G)	30 (A->G)	21	10	11	13, 15 (T->C)	13 (C->T)	11	12
African American	OT05898	15	12, 13	13	17	30	22 (G->A)	10	12	13, 14 (T->C)	16	10	12
African American	OT05899	15	16, 17	13	18 (A->G)	31 (A->G)	22 (A->G)	10	11	13, 14 (T->C)	14 (C->T)	11	12
African American	OT05901	14	11, 14	13	16	29	23	11	13	13, 14 (T->C)	15	12	11
African American	PT84214	17	17, 18	14	17 (A->G)	31 (A->G)	21	10	11	13 (T->C), 15	14 (C->T)	11	12
African American	PT84215	15	16, 17	13	17 (A->G)	30 (A->G)	21	10	11	13, 13 (T->C)	14 (C->T)	11	11
African American	PT84216	13	16, 16	13	17 (A->G)	30 (A->G)	24	10	13	13, 14 (T->C)	15	10	12
African American	PT84222	14	13, 14	12	16	28	22	10	11	13, 14 (T->C)	16	10	11
African American	PT84223	15	15, 15	13	18 (A->G)	31 (A->G)	21	10	12	13, 14 (T->C)	14 (C->T)	11	12
African American	PT84224	15	16, 17	13	17 (A->G)	30 (A->G)	21	11	11	13, 15 (T->C)	14 (C->T)	11	12
African American	PT84225	15	13, 15	13	18 (A->G)	31 (A->G)	21	10	11	13, 15 (T->C)	14 (C->T)	11	11
African American	PT84226	15	16, 17	14	17 (A->G)	31 (A->G)	20	10	11	13, 14 (T->C)	14 (C->T)	11	13
African American	PT84227	15	16, 16	13	16	29	24	10	12	15, 15 (T->C)	15	10	12
African American	PT84228	14	12, 15	13	15	28	24	11	13	13, 15 (T->C)	15	12	11
African American	PT84230	16	16, 17	14	18 (A->G)	32 (A->G)	21	11	11	13, 13 (T->C)	14 (C->T)	11	12
African American	PT84231	16	18, 18	12	18 (A->G)	30 (A->G)	21	10	11	13 (T->C), 15	14 (C->T)	11	12
African American	PT84232	15	11, 15	13	16	29	25	10	13	12, 14 (T->C)	15	12	13
African American	PT84234	15	14, 15	13	18 (A->G)	31 (A->G)	21	10	11	13, 17 (T->C)	14 (C->T)	11	11
African American	PT84236	14	11, 14	13	16	29	24	11	13	13, 13 (T->C)	14	12	12
African American	PT84239	15	16, 16	13	17 (A->G)	30 (A->G)	21	10	11	13, 14 (T->C)	14 (C->T)	11	12
African American	PT84240	16	11, 12	13	18 (A->G)	31 (A->G)	24	10	7	13, 14 (T->C)	14	10	12
African American	PT84241	16	11, 13	13	17	30	25	10	11	13, 14 (T->C)	14	11	12
African American	PT84242	15	15, 18	13	18 (A->G)	31 (A->G)	21	10	11	14, 14 (T->C)	14 (C->T)	11	11
African American	PT84243	16	16, 16	12	18 (A->G)	30 (A->G)	21	10	11	13 (T->C), 14	14 (C->T)	11	12
Caucasian	BC11352	15	15, 17	14	16	30	22	10	11	12, 14 (T->C)	14	9	11
Caucasian	MT94859	15	15, 15	14	17	31	23	10	12	12 (T->C), 14	14	10	11
Caucasian	MT94866	14	11, 16	13	17	30	24	11	15	12, 15 (T->C)	15	12	12
Caucasian	MT94868	14	11, 14	13	16	29	23	11	13	13, 14 (T->C)	16	12	12
Caucasian	MT94869	14	11, 14	13	17	30	24	10	13	13, 15 (T->C)	15	12	12
Caucasian	MT94875	14	11, 14	13	16	29	24	11	13	13, 14 (T->C)	15	12	11
Caucasian	MT97172	16	13, 18	12	16	28	24	10	11	12, 12 (T->C)	16	9	11
Caucasian	UT57300	15	14, 14	13	17	30	23	10	12	13 (T->C), 14	14	10	11
Caucasian	UT57301	14	12, 14	13	15	28	24	11	13	13, 14 (T->C)	15	12	12
Caucasian	UT57302	14	11, 15	13	16	29	24	11	13	13, 15 (T->C)	15	12	12
Caucasian	UT57303	15	14, 17	12	16	28	24	10	11	12, 15 (T->C)	16	9	12
Caucasian	UT57310	15	11, 14	12	19	31	25 (A->C)	10	11	13, 15 (T->C)	14	11	10
Caucasian	UT57312	14	11, 15	13	16	29	24	11	13	13, 13 (T->C)	15	12	13
Caucasian	UT57317	16	13, 15	13	17	30	23	9	11	12, 15 (T->C)	14	9	12
Caucasian	UT57318	14	11, 14	14	16	30	24	11	13	13, 13 (T->C)	15	12	12
Caucasian	WA29584	14	11, 15	13	16	29	24	11	13	13, 13 (T->C)	15	12	12
Caucasian	WA29594	13	17, 18	14	17 (A->G)	31 (A->G)	25	9	11	13, 14 (T->C)	14	10	13
Caucasian	WA29612	14	12, 14	13	16	29	23	11	13	12, 13 (T->C)	15	12	12
Caucasian	WT51342	14	11, 14	14	16	30	24	11	13	13, 15 (T->C)	15	12	12
Caucasian	WT51343	14	11, 15	14	17	31	23	11	13	13, 13 (T->C)	14	12	12
Caucasian	WT51345	14	11, 14	13	17	30	23	12	13	13, 15 (T->C)	15	12	12
Caucasian	WT51354	14	12, 14	14	17	31	24	9	13	13, 14 (T->C)	15	12	12
Caucasian	WT51355	15	11, 14	14	16	30	24	11	13	13, 15 (T->C)	15	12	13
Caucasian	WT51358	16	11, 14	13	16	29	23	10	13	13, 14 (T->C)	16	11	12
Caucasian	WT51359	14	11, 14	13	16	29	24	11	13	13, 15 (T->C)	15	12	12
Caucasian	WT51362	14	11, 14	13	16	29	23	11	13	13, 13 (T->C)	15	12	12
Caucasian	WT51373	14	11, 14	14	16	30	25	11	13	12 (T->C), 13	15	12	12
Caucasian	WT51378	14	11, 14	13	16	29	23	11	13	12, 14 (T->C)	15	12	12
Caucasian	WT51381	14	11, 14	13	16	29	24	11	11	13, 13 (T->C)	14	12	13
Caucasian	WT51386	15	13, 17	12	16	28	24	10	11	12, 15 (T->C)	16	9	12
Caucasian	ZT81387	15	11, 14	13	17 (G->A)	30 (A->G)	25 (A->C)	10	11	13, 13 (T->C)	14	11	10

Concordant with truth data
Concordant with base allele call(s) in truth data, but also contained SNP(s)
No truth data available

***** The deduced allele call for DYS389II was derived by adding the allele numbers for DYS389I and DYS389II-1 The concordance of the allele for DYS389II-1 was deduced by the allele being equal to the truth data for DYS389II minus DYS389I

¥ Locus DYS393 produced two products in all samples. One had a T->C SNP in every case and the other did not. The one without a T->C SNP was concordant in every case with the truth data.

Figure 45, part 1. NIST sample Y-STR typing results for African American and Caucasian populations.

Population	Sample	DYS19	DYS385a/b	DYS389I	DYS389II-1	Deduced* DYS389II	DYS390	DYS391	DYS392	DYS393¥	DYS437	DYS438	DYS439
Hispanic	GT37778	16	16, 17	13	17 (A->G)	30 (A->G)	21	10	11	13, 13 (T->C)	14 (C->T)	11	12
Hispanic	GT37812	15	11, 14	14	16	30	23	11	14	12 (T->C), 13	15	12	11
Hispanic	GT37828	15	15, 16 (G->A + G->C)	13	17 (G->A)	30 (G->A)	23	11	13	13, 15 (T->C)	13	9	11
Hispanic	GT37862	13	14, 18	13	17	30	25	10	16	13, 15 (T->C)	14	11	11
Hispanic	GT37864	14	12, 16	13	16	29	24	11	13	13, 13 (T->C)	15	12	13
Hispanic	GT37869	14	11, 15	13	16	29	24	11	14	13, 14.3 (T->C)	15	10	13
Hispanic	GT37888	13	13, 14	14	16 (A->G)	30 (A->G)	24	9	11	13, 14 (T->C)	14	10	10
Hispanic	GT37900	16	13, 16	13	16	29	23	9	11	12, 13 (T->C)	14	9	13
Hispanic	GT37913	15	16, 18	12	16	28	24	10	11	12, 13 (T->C)	14	9	13
Hispanic	JT52076	14	13, 14	12	16	28	22	10	11	13, 16 (T->C)	17 (A->G)	10	11
Hispanic	OT07280	14	11, 14	12	17	29	24	11	11	13, 13 (T->C)	15	12	13
Hispanic	PT85612	15	16, 16	13	18 (A->G)	31 (A->G)	21	11	11	13, 15 (T->C)	14 (C->T)	11	11
Hispanic	PT85658	14	12 (A->G), 14	13	16	29	24	11	13	13, 14 (T->C)	15	12	12
Hispanic	TT51399	13	14, 17	13	17 (G->A)	30 (A->G)	24	10	16	13, 15 (T->C)	14	11	12
Hispanic	TT51407	15	16, 19	13	18 (A->G + C->G)	31 (A->G)	23	10	11	13 (T->C), 14	14	11	13
Hispanic	TT51422	16	11, 13	12	17	29	25	10	11	13, 15 (T->C)	14	11	10
Hispanic	TT51435	15	13, 15	12	18	30	21	10	11	13 (T->C), 15	16	10	12
Hispanic	TT51483	16	12, 12	13	15	28	23	10	11	13, 15 (T->C)	14	10	13
Hispanic	TT51511	15	11, 14	13	16	29	23	11	13	13, 14 (T->C)	15	12	13
Hispanic	TT51530	13	15, 18	12	16	28	23	10	13	14, 14 (T->C)	14	11	13
Hispanic	ZT80731	16	16, 16	14	17 (A->G)	31 (A->G)	21 (C->A)	10	11	13, 15 (T->C)	14 (C->T)	11	13
Hispanic	ZT80737	14	11, 13	13	16	29	25	11	13	13, 15 (T->C)	15	12	12
Hispanic	ZT80782	14	10, 14	14	16	30	24	11	13	13, 14 (T->C)	15	13	11
Hispanic	ZT80786	14	13, 18	12	18	30	23	11	11	12, 12 (T->C)	14	10 (A->C)	11
Hispanic	ZT80815	14	13, 15	12	16	28	23 (A->G)	11	11	13, 14 (T->C)	16	10	11
Hispanic	ZT80826	14	11, 14	14	16	30	23	10	13	12 (T->C), 13	14	12	12
Hispanic	ZT80863	17	12, 12	13	15	28	23	10	11	13, 15 (T->C)	15	10	12
Hispanic	ZT80865	15	11, 14	13	17	30	24	11	13	13, 15 (T->C)	15	12	12
Hispanic	ZT80869	13	13, 15	12	16	28	24 (A->G)	10	11	13, 13 (T->C)	16	10	11
Hispanic	ZT80870	15	13, 16	12	17	29	24	10	11	12, 14 (T->C)	16	9	12
Hispanic	ZT80925	13	15, 18	13	17 (A->G)	30 (A->G)	24	10	11	13, 14 (T->C)	14	10	12
Hispanic	ZT80932	14	11, 14	13	16	29	24	11	13	12, 13 (T->C)	15	12	12

Concordant with truth data
Concordant with base allele call(s) in truth data, but also contained SNP(s)
No truth data available

***** The deduced allele call for DYS389II was derived by adding the allele numbers for DYS389I and DYS389II-1
The concordance of the allele for DYS389II-1 was deduced by the allele being equal to the truth data for DYS389II minus DYS389I

¥ Locus DYS393 produced two products in all samples. One had a T->C SNP in every case and the other did not. The one without a T->C SNP was concordant in every case with the truth data.

Figure 45, part 2. NIST sample Y-STR typing results for samples from the Hispanic population.

panel testing, however, the genotypes for DYS393 did not all consist of two same-length alleles. In fact, 78% of the samples had two different-length alleles at DYS393. Every sample had one allele at DYS393 that was consistent with a known allele with a T→C SNP in it, and in every case the other allele was consistent with a non-polymorphic allele. For these 95 samples, the non-polymorphic allele was consistent with the truth data in all 92 cases where there was truth data. The initial interpretation of this result was that additional individual-differentiating information obtained with the second DYS393 allele could be exploited by inclusion of primer pair 4602 in our final primer panel. It appears, however, that the additional alleles are the result of amplifying the homolog of DYS393 from the X-chromosome[68, 69], and this primer pair was replaced in subsequent panels.

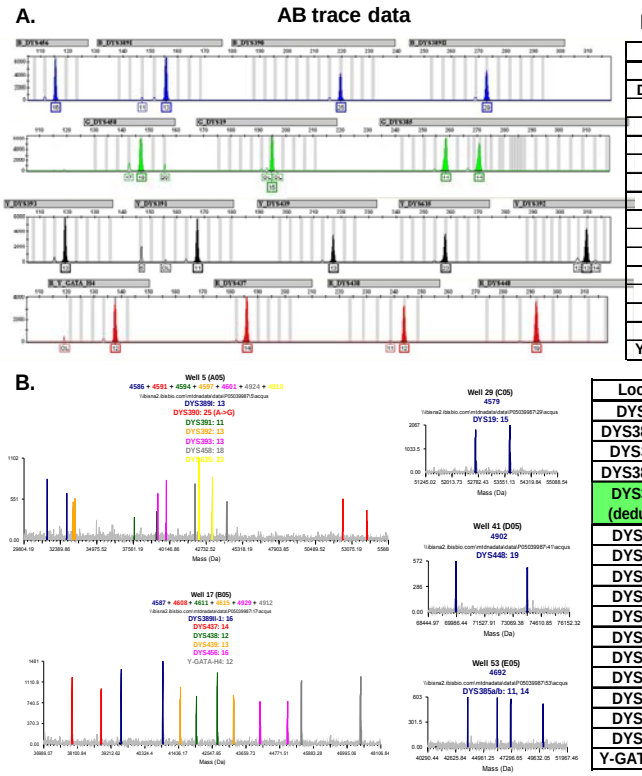
Table 31. Frequency of polymorphic alleles observed in 95 population samples from NIST.

Locus	Number of different alleles with SNPs seen	Percentage of samples with variant alleles
DYS385a/b	3	3.2
DYS389II	5	33.7
DYS390	6	7.4
DYS437	3	25.3
DYS438	1	1.1

In addition to being concordant with existing truth data, polymorphisms were revealed in five of the ten loci tested (Table 31). The highest frequency of polymorphisms from this initial small test set was seen in DYS389II. All of these were in the 5′ repeat region of the double locus (no polymorphisms were seen in DYS389I). For the 92 samples having truth data, the sum of the base allele numbers for DYS389I and

DYS389II-1 was the same as the truth data allele number for DYS389I/II, suggesting that the strategy of splitting DYS389I/II into two separately analyzed products will still remained backwards-compatible with existing databases because the sum of the two alleles can be used to compare to existing genotypes for DYS389I/II. Truth data for the NIST population samples referenced in Figure 45 can be found at http://www.cstl.nist.gov/strbase/NISTpopdata/YfilerNISTdata.xls.

To assess the ability to generate profiles concordant with existing "Gold standard" technology with the full set of 16 markers used in the final assay, 34 blood-derived male DNA samples were amplified at Ibis with the AB Y-Filer™ system and analyzed on an AB 310 single-capillary instrument in-house. The same 34



Final genotype

Locus	Allele
DYS19	15
DYS385a/b	11,14
DYS389I	13
DYS389II	29
DYS390	25
DYS391	11
DYS392	13
DYS393	13
DYS437	14
DYS438	12
DYS439	13
DYS448	19
DYS456	16
DYS458	18
DYS635	23
Y-GATA-H4	12

Locus	Allele
DYS19	15
DYS385a/b	11, 14
DYS389I	13
DYS389II-1	16
DYS389II (deduced)	29
DYS390	25 (A->G)
DYS391	11
DYS392	13
DYS393	13
DYS437	14
DYS438	12
DYS439	13
DYS448	19
DYS456	16
DYS458	18
DYS635	23
Y-GATA-H4	12

Figure 46. Data generated with 1 ng/reaction blood sample 55-25307 using the AB Y-Filer system (panel A) and the Ibis system (panel B). Note that the allele assignment for locus DYS389II is deduced from DYS389I and the left side of DYS389II in the Ibis system and that the deduced allele call is concordant with that observed using the AB system. Also note that allele 25 at locus DYS390 in the Ibis data has an A→G SNP in it.

Table 32. Final allele calls for 34 blood-derived DNA samples using the AB Y-Filer system with an AB 310 instrument.

Sample Name	DYS19	DYS385a/b	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
55-24133	14	11, 14	13	29	24	11	13	12	15	13	12	19	15	16	23	13
55-24336	16	15, 16	13	30	21	10	11	14	14	11	13	20	16	17	23	11
55-24338	14	11, 13	13	29	24	10	14	13	15	12	12	18	17	17	23	12
55-24413	14	11, 15	13	29	24	11	13	13	15	13	11	19	16	17	23	11
55-24622	13	16, 18	13	31	24	10	11	13	14	10	12	20	18	17	22	12
55-24907	14	17, 18	12	29	24	10	11	14	14	10	11	20	15	16	21	12
55-24916	16	11, 14	13	30	25	10	11	13	15	11	10	19	16	15	25	11
55-25006	14	11, 13	13	29	25	11	14	13	15	12	12	18	15	16	24	12
55-25110	15	11, 14	13	30	26	11	13	13	15	12	11	20	15	17	24	13
55-25113	14	11, 13	13	29	24	10	13	13	14	10	11	19	16	17	23	12
55-25185	16	13, 16	13	29	24	9	11	12	14	9	11	21	17	14	22	12
55-25188	15	11, 15	13	29	24	11	13	13	15	12	12	19	16	17	23	12
55-25192	14	11, 15	13	30	24	11	13	14	15	12	13	19	16	17	23	12
55-25193	14	11, 15	13	29	24	11	13	14	15	12	13	19	15	19	23	12
55-25236	14	11, 13	13	29	24	10	13	13	15	12	11	19	16	17	24	12
55-25238	14	11, 13	13	29	25	11	14	13	14	12	12	18	17	16	24	12
55-25290	14	11, 14	14	30	24	11	13	13	15	12	12	18	16	17	23	12
55-25307	15	11, 14	13	29	25	11	13	13	14	12	13	19	16	18	23	12
55-25356	16	11, 14	13	29	25	10	11	13	14	11	11	20	17	16	23	12
55-25364	15	13, ---	12	28	23	10	14	13	14	10	11	18	15	16	20	12
55-25367	13	13, 14	14	30	24	10	11	13	14	10	10	20	15	17	21	12
55-25381	14	11, 14	13	28	24	10	13	14	15	12	12	19	16	17	23	13
55-25445	15	14, 16	14	31	23	10	12	14	14	10	10	21	14	14	21	12
55-25456	15	16, 17	14	31	20	10	11	13	14	11	13	20	17	18	20	13
55-25460	14	11, 14	13	29	25	10	13	13	15	12	11	18	15	17	23	11
55-25502	14	17, 18	12	29	24	10	11	14	14	10	11	20	15	16	21	12
55-25577	14	11, 13	13	29	25	11	14	13	15	12	12	18	15	16	24	12
55-25578	14	17, 19	14	32	25	10	11	13	14	10	11	20	16	17	21	12
55-25597	14	11, 15	13	29	24	10	14	12	15	12	12	19	16	18	23	11
55-25600	16	15, ---	13	29	23	10	12	14	15	10	11	20	17	17	21	11
55-25602	14	11, 14	13	29	24	11	13	13	15	12	12	19	17	17	23	12
55-SMPL11	14	11, 15	13	30	24	10	13	13	15	12	11	19	16	20	24	13
SC35495-31	14	11, 14	13	29	24	11	13	13	15	12	13	19	16	16	24	12
SC48046	14	13, 14	12	29	23	10	11	14	16	10	11	20	14	16	23	11

Table 33. Allele calls for 34 blood-derived DNA samples using the Ibis assay system. Allele assignments for DYS389II were deduced from the sum of the allelic numbers for DYS389I and DYS389II-1, and in each case they were concordant in nominal assignment to data obtained with the Y-Filer system.

Sample	DYS19	DYS385a/b	DYS389I	DYS389II (deduced)	DYS389II-1	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS448	DYS456	DYS458	DYS635	Y-GATA-H4
55-24133	14	11, 14	13	29	16	24	11	13	12	15	13	12	19	15	16	23	13
55-24336	16	15, 16	13	30 (A->G)	17 (A->G)	21	10	11	14	14 (C->T)	11	13	20	16	17	23 (2C->2G)	11
55-24338	14	11, 13	13	29	16	24	10	14	13	15	12	12	18	17	17	23	12
55-24413	14	11, 15	13	29	16	24	11	13	13	15	13	11	19	16	17	23	11
55-24622	13	16, 18	13	31 (A->G)	18 (A->G)	24	10	11	13	14	10	12	20	18	17	22 (2C->2G)	12
55-24907	14	17, 18	12	29 (A->G)	17 (A->G)	24	10	11	14	14	10	11	20	15	16	21 (2C->2G)	12
55-24916	16	11, 14	13	30	17	25 (A->C)	10	11	13 (A->C)	15	11	10	19	16	15	25	11
55-25006	14	11, 13	13	29	16	25	11	14	13	15	12	12	18	15	16	24	12
55-25110	15	11, 14	13	30	17	26	11	13	13	15	12	11	20	15	17	24	13
55-25113	14	11, 13	13	29	16	24	10	13	13	14	10	11	19	16	17	23	12
55-25185	16	13, 16	13	29	16	24	9	11	12	14	9	11	21	17	14 (A->G)	22 (2C->2G)	12
55-25188	15	11, 15	13	29	16	24	11	13	13	15	12	12	19	16	17	23	12
55-25192	14	11, 15	13	30	17	24	11	13	14	15	12	13	19	16	17	23	12
55-25193	14	11, 15	13	29	16	24	11 (A->G)	13	14	15	12	13	19	15	19	23	12
55-25236	14	11, 13 (G->A)	13	29	16	24	10	13	13	15	12	11	19	16	17	24	12
55-25238	14	11, 13	13	29	16	25	11	14	13	14	12	12	18	17	16	24	12
55-25290	14	11, 14	14	30	16	24	11	13	13	15	12	12	18	16	17	23	12
55-25307	15	11, 14	13	29	16	25 (A->G)	11	13	13	14	12	13	19	16	18	23	12
55-25356	16	11, 14	13	29	16	25	10	11	13 (A->C)	14	11	11	20	17	16	23	12
55-25364	15	13, ---	12	28 (A->G)	16 (G->A)	23	10	14	13	14	10	11	18	15	16	20 (2C->2G)	12
55-25367	13	13, 14	14	30 (A->G)	16 (A->G)	24	10	11	13	14	10	10	20	15	17	21 (2C->2G)	12
55-25381	14	11, 14	13	28	15	24	10	13	14	15	12	12	19	16	17	23	13
55-25445	15	14, 16	14	31	17	23	10	12	14	14	10	10	21	14	14	21 (2C->2G)	12
55-25456	15	16, 17	14	31 (A->G)	17 (A->G)	20	10	11	13	14 (C->T)	11	13	20	17	18	20 (2C->2G)	13
55-25460	14	11, 14	13	29	16	25	10	13	13	15	12	11	18	15	17	23	11
55-25502	14	17, 18	12	29 (A->G)	17 (A->G)	24	10	11	14	14	10	11	20	15	16	21 (2C->2G)	12
55-25577	14	11, 13	13	29	16	25	11	14	13	15	12	12	18	15	16	24	12
55-25578	14	17, 19	14	32 (A->G)	18 (A->G)	25	10	11	13	14	10	11	20	16	17	21 (2C->2G)	12
55-25597	14	11, 15	13	29	16	24	10	14	12	15	12	12	19	16	18	23	11
55-25600	16	15, 15 (G->A)	13	29	16	23	10	12	14	15	10 (C->A)	11	20	17	17	21 (2C->2G)	11
55-25602	14	11, 14	13	29	16	24	11	13	13	15	12	12	19	17	17	23	12
55-SMPL11	14	11, 15	13	30	17	24	10	13	13	15	12	11	19	16	20	24	13
SC35495-31	14	11, 14	13	29	16	24	11	13	13	15	12	13	19	16	16	24	12
SC48046	14	13, 14	12	29	17	23	10	11	14	16	10	11	20	14	16	23 (2C->2G)	11

samples were then analyzed using the preliminary Ibis kit consisting of one six-plex, one seven-plex and three single-plex reactions (Figure 29, layout option 1). Figure 46 outlines an example of comparison of the data generated with the AB system (panel A) to the data generated with the Ibis system for the same sample (panel B).

Full Profiles obtained with the Y-Filer system are shown in Table 32. Profiles obtained using the Ibis system are shown in Table 33. All nominal allele calls were concordant, and all deduced DYS389II assignments, which were obtained by simply adding the allele numbers for DYS389I and DYS389II-1, were concordant with DYS389II assignments made with the Y-Filer system. Note the case in sample 55-25600, where the "Gold standard" genotype for DYS385a/b is homozygous 15. Examination of the mass-spectrometry data, however, reveals this sample to be heterozygous with one nominal allele 15 and one allele 15 with a G→A SNP.

Specific Aim 3: Characterize polymorphisms in core autosomal STR and Y-STR markers

In collaboration with Art Eisenberg and John Planz at the University at North Texas Health Sciences Center (UNTHSC), we have surveyed polymorphisms in core autosomal STR loci for a set of 847 samples, which, combined with the 95 samples from NIST, consists of 297 Caucasian, 332 African American and 313 Hispanic samples. Allele frequencies, including polymorphisms, are shown in Table 34 for these 942 samples.

Table 34, part 1. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
CSF1PO	11	177	154	181	29.8	23.2	28.9
	13	45	38	38	7.6	5.7	6.1
	10	156	193	158	26.3	29.1	25.2
	12	195	170	223	32.8	25.6	35.6
	7	0	41	4	0.0	6.2	0.6
	6	0	1	0	0.0	0.2	0.0
	8	0	38	3	0.0	5.7	0.5
	9	14	25	14	2.4	3.8	2.2
	11S2	0	1	0	0.0	0.2	0.0
	12S2	0	1	0	0.0	0.2	0.0
	14	5	2	4	0.8	0.3	0.6
	15	2	0	1	0.3	0.0	0.2
D13S317	9	36	15	125	6.1	2.3	20.0
	12S9	73	80	63	12.3	12.0	10.1
	11	71	111	68	12.0	16.7	10.9
	12	115	205	94	19.4	30.9	15.0
	8	58	19	53	9.8	2.9	8.5
	11S9	109	88	50	18.4	13.3	8.0
	13	42	84	63	7.1	12.7	10.1
	14	29	18	33	4.9	2.7	5.3
	13S9	17	23	16	2.9	3.5	2.6
	10	31	8	22	5.2	1.2	3.5
	10S9	6	8	36	1.0	1.2	5.8
	14S9	6	3	1	1.0	0.5	0.2
	9S9	0	1	1	0.0	0.2	0.2
	15	0	1	0	0.0	0.2	0.0
	13S4	1	0	0	0.2	0.0	0.0
	15S9	0	0	1	0.0	0.0	0.2
D3S1358	16S1.2	13	113	12	2.2	17.0	1.9
	17S1	63	83	31	10.6	12.5	5.0
	15S1.2	21	108	19	3.5	16.3	3.0
	16S1	88	65	102	14.8	9.8	16.3
	14S1	73	48	65	12.3	7.2	10.4
	17	43	60	51	7.2	9.0	8.1
	16	44	37	33	7.4	5.6	5.3
	18	67	26	54	11.3	3.9	8.6
	15S1	148	61	222	24.9	9.2	35.5
	13S1.2	0	3	0	0.0	0.5	0.0
	12S1.2	0	2	0	0.0	0.3	0.0
	15	6	13	6	1.0	2.0	1.0
	17S1.2	4	19	7	0.7	2.9	1.1
	13S1	1	1	6	0.2	0.2	1.0
	19	6	1	3	1.0	0.2	0.5
	18S1.2	0	4	1	0.0	0.6	0.2
	18S1	8	6	7	1.3	0.9	1.1
	14S1.2	0	10	3	0.0	1.5	0.5
	14	1	1	2	0.2	0.2	0.3
	15.1S1S10	0	1	0	0.0	0.2	0.0
	15.2S1	0	1	0	0.0	0.2	0.0
	17S3	0	1	0	0.0	0.2	0.0
	11S1	3	0	1	0.5	0.0	0.2
	19S1	1	0	0	0.2	0.0	0.0
	16S2	1	0	0	0.2	0.0	0.0
	17S2	3	0	1	0.5	0.0	0.2

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D21S11	29	105	111	101	17.7	16.7	16.1
	31	26	49	37	4.4	7.4	5.9
	31.2	55	34	59	9.3	5.1	9.4
	28	86	157	65	14.5	23.6	10.4
	30S2	69	94	127	11.6	14.2	20.3
	27S2	18	16	7	3.0	2.4	1.1
	33.2	15	21	23	2.5	3.2	3.7
	37S2.2	0	1	0	0.0	0.2	0.0
	32.2	63	45	82	10.6	6.8	13.1
	27	1	15	2	0.2	2.3	0.3
	35S2.2	0	3	1	0.0	0.5	0.2
	29S1	29	6	18	4.9	0.9	2.9
	29S2	1	13	0	0.2	2.0	0.0
	30S2.2	0	3	1	0.0	0.5	0.2
	30	72	23	46	12.1	3.5	7.3
	32S2	2	14	4	0.3	2.1	0.6
	30.2	16	7	8	2.7	1.1	1.3
	35.2	3	2	0	0.5	0.3	0.0
	31S1	14	7	9	2.4	1.1	1.4
	32	4	3	2	0.7	0.5	0.3
	25.2	1	1	0	0.2	0.2	0.0
	33S2	0	1	0	0.0	0.2	0.0
	35S7	0	10	0	0.0	1.5	0.0
	36S2.2	0	2	0	0.0	0.3	0.0
	34.2	0	4	2	0.0	0.6	0.3
	34S2.2	0	1	0	0.0	0.2	0.0
	35	1	4	0	0.2	0.6	0.0
	33.1	0	2	1	0.0	0.3	0.2
	33	0	2	1	0.0	0.3	0.2
	29.2S2	2	1	0	0.3	0.2	0.0
	33.2S2	0	1	1	0.0	0.2	0.2
	34	0	2	1	0.0	0.3	0.2
	34S2S11	0	1	0	0.0	0.2	0.0
	31.2S1	2	1	5	0.3	0.2	0.8
	26	0	1	2	0.0	0.2	0.3
	36	0	1	0	0.0	0.2	0.0
	36S2	0	1	0	0.0	0.2	0.0
	28S1	1	1	1	0.2	0.2	0.2
	36S2S10	0	1	0	0.0	0.2	0.0
	37S2.3S1	0	1	0	0.0	0.2	0.0
	37S2.3	0	1	0	0.0	0.2	0.0
	29.3	1	0	0	0.2	0.0	0.0
	32.2S1	1	0	9	0.2	0.0	1.4
	30.2S1	4	0	3	0.7	0.0	0.5
	33S1	1	0	2	0.2	0.0	0.3
	29.2	1	0	0	0.2	0.0	0.0
	30S2S11	0	0	2	0.0	0.0	0.3
	33.2S1	0	0	3	0.0	0.0	0.5
	28.2	0	0	1	0.0	0.0	0.2
AMEL	X	428	459	463	72.1	69.1	74.0
	Y	166	205	163	27.9	30.9	26.0

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

Table 34, part 2. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D18S51	15	87	109	112	14.6	16.4	17.9
	19	30	54	16	5.1	8.1	2.6
	17	70	105	93	11.8	15.8	14.9
	18	38	66	41	6.4	9.9	6.5
	10	8	3	0	1.3	0.5	0.0
	16	75	117	60	12.6	17.6	9.6
	20	12	26	13	2.0	3.9	2.1
	24	1	3	2	0.2	0.5	0.3
	12	88	54	69	14.8	8.1	11.0
	14.2S4	0	2	0	0.0	0.3	0.0
	15.2	0	2	0	0.0	0.3	0.0
	22	2	7	10	0.3	1.1	1.6
	21	3	9	6	0.5	1.4	1.0
	14	87	47	106	14.6	7.1	16.9
	13	80	34	74	13.5	5.1	11.8
	9	0	2	0	0.0	0.3	0.0
	17S4	0	1	2	0.0	0.2	0.3
	16S4	0	1	0	0.0	0.2	0.0
	14S7	3	4	4	0.5	0.6	0.6
	18S4	0	2	1	0.0	0.3	0.2
	21.2	0	1	0	0.0	0.2	0.0
	12S4	0	2	0	0.0	0.3	0.0
	11	8	2	9	1.3	0.3	1.4
	13S4	0	1	0	0.0	0.2	0.0
	19S4	0	1	0	0.0	0.2	0.0
	13.2S4	1	3	0	0.2	0.5	0.0
	11S4	0	1	0	0.0	0.2	0.0
	20S4	0	4	0	0.0	0.6	0.0
	23	0	1	3	0.0	0.2	0.5
	15S7	1	0	1	0.2	0.0	0.2
	25	0	0	3	0.0	0.0	0.5
	15S4.2	0	0	1	0.0	0.0	0.2
D7S820	10	130	191	144	21.9	28.8	23.0
	9	76	101	55	12.8	15.2	8.8
	11	113	119	174	19.0	17.9	27.8
	13	14	9	20	2.4	1.4	3.2
	12	70	60	86	11.8	9.0	13.7
	8	98	150	71	16.5	22.6	11.3
	12S10	21	11	23	3.5	1.7	3.7
	14	6	3	2	1.0	0.5	0.3
	11S10	13	3	16	2.2	0.5	2.6
	10S10	32	8	20	5.4	1.2	3.2
	7	12	6	9	2.0	0.9	1.4
	13S10	5	2	2	0.8	0.3	0.3
	11S2	0	1	0	0.0	0.2	0.0
	9S4	2	0	0	0.3	0.0	0.0
	9S10	1	0	1	0.2	0.0	0.2
	7S10	1	0	0	0.2	0.0	0.0
	10.3	0	0	3	0.0	0.0	0.5

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
D5S818	12	163	182	128	27.4	27.4	20.4
	13	56	120	61	9.4	18.1	9.7
	11	205	126	272	34.5	19.0	43.5
	12S8	65	62	31	10.9	9.3	5.0
	8S8	0	50	3	0.0	7.5	0.5
	11S8	24	24	16	4.0	3.6	2.6
	13S8	21	34	10	3.5	5.1	1.6
	9	0	1	0	0.0	0.2	0.0
	10S8	6	4	9	1.0	0.6	1.4
	9S8	20	12	31	3.4	1.8	5.0
	10	20	24	21	3.4	3.6	3.4
	14	6	10	6	1.0	1.5	1.0
	13S6	1	8	3	0.2	1.2	0.5
	15S6	0	1	0	0.0	0.2	0.0
	7	1	1	33	0.2	0.2	5.3
	14S6	0	2	0	0.0	0.3	0.0
	14S8	2	2	1	0.3	0.3	0.2
	15	2	1	1	0.3	0.2	0.2
	8	2	0	0	0.3	0.0	0.0
D8S1179	11	44	22	38	7.4	3.3	6.1
	14S2	96	219	138	16.2	33.0	22.0
	13S2	160	120	137	26.9	18.1	21.9
	16S2.2	0	10	0	0.0	1.5	0.0
	12S2	4	38	13	0.7	5.7	2.1
	15S2	53	107	60	8.9	16.1	9.6
	10	55	8	68	9.3	1.2	10.9
	12	91	33	66	15.3	5.0	10.5
	15S2.2	0	18	0	0.0	2.7	0.0
	17S2	2	3	1	0.3	0.5	0.2
	13	42	26	52	7.1	3.9	8.3
	16S2	15	34	13	2.5	5.1	2.1
	14	15	8	23	2.5	1.2	3.7
	11S2	0	6	1	0.0	0.9	0.2
	17S2.2	0	4	0	0.0	0.6	0.0
	8	7	4	4	1.2	0.6	0.6
	17S2.3	0	1	0	0.0	0.2	0.0
	9	7	2	2	1.2	0.3	0.3
	13S2.2	0	1	0	0.0	0.2	0.0
	14S2S5	2	0	0	0.3	0.0	0.0
	14S2.2S12	1	0	0	0.2	0.0	0.0
	13S2S5	0	0	6	0.0	0.0	1.0
	16	0	0	1	0.0	0.0	0.2
	15	0	0	2	0.0	0.0	0.3
	18S2	0	0	1	0.0	0.0	0.2
TPOX	8	308	234	341	51.9	35.2	54.5
	9	62	134	43	10.4	20.2	6.9
	11	143	126	142	24.1	19.0	22.7
	7	1	14	3	0.2	2.1	0.5
	10	52	78	25	8.8	11.7	4.0
	12	27	15	70	4.5	2.3	11.2
	6	1	62	1	0.2	9.3	0.2
	13	0	1	1	0.0	0.2	0.2

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

Table 34, part 3. Observed frequency of each allele by population in 297 Caucasian, 332 African American and 313 Hispanic samples from UNTHSC and NIST.

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
vWA	17	139	107	151	23.4	16.1	24.1
	18	118	88	102	19.9	13.3	16.3
	15S1	59	32	37	9.9	4.8	5.9
	20	5	6	6	0.8	0.9	1.0
	15	10	96	33	1.7	14.5	5.3
	17S1	10	28	12	1.7	4.2	1.9
	18S1	4	11	2	0.7	1.7	0.3
	20S2.2	0	1	0	0.0	0.2	0.0
	16	93	116	187	15.7	17.5	29.9
	16S1	21	56	14	3.5	8.4	2.2
	14S2S4.2	49	23	32	8.2	3.5	5.1
	14S1S4	16	6	5	2.7	0.9	0.8
	14S4	3	21	3	0.5	3.2	0.5
	19S2.2	0	7	0	0.0	1.1	0.0
	20S2	1	6	0	0.2	0.9	0.0
	13S3	1	10	0	0.2	1.5	0.0
	19	52	27	35	8.8	4.1	5.6
	18.3	1	1	0	0.2	0.2	0.0
	19S2	1	2	2	0.2	0.3	0.3
	18S2.2	0	2	0	0.0	0.3	0.0
	17S2S11	1	1	0	0.2	0.2	0.0
	19S1	1	3	0	0.2	0.5	0.0
	18S2	2	4	1	0.3	0.6	0.2
	20S2.3	0	1	0	0.0	0.2	0.0
	11	0	5	0	0.0	0.8	0.0
	21S2	0	1	1	0.0	0.2	0.2
	12	0	1	0	0.0	0.2	0.0
	13S1S3	1	1	0	0.2	0.2	0.0
	21S2S2	0	1	0	0.0	0.2	0.0
	17S2	1	0	0	0.2	0.0	0.0
	21	1	0	0	0.2	0.0	0.0
	18S10	2	0	1	0.3	0.0	0.2
	15S2S4.2	1	0	1	0.2	0.0	0.2
	20S10	1	0	0	0.2	0.0	0.0
	18S2.2S11	0	0	1	0.0	0.0	0.2
D16S539	9	68	154	58	11.4	23.2	9.3
	12	178	119	174	30.0	17.9	27.8
	11	179	185	187	30.1	27.9	29.9
	14	17	11	10	2.9	1.7	1.6
	13	108	94	88	18.2	14.2	14.1
	10	33	76	103	5.6	11.4	16.5
	8	10	23	3	1.7	3.5	0.5
	7	0	1	0	0.0	0.2	0.0
	10S9.2	0	1	0	0.0	0.2	0.0
	9S2	1	0	0	0.2	0.0	0.0
	8S5	0	0	3	0.0	0.0	0.5

		Count			Percentage		
Locus	Allele	Caucasian	African American	Hispanic	Caucasian	African American	Hispanic
FGA	23	81	119	83	13.6	17.9	13.3
	31.2	1	5	0	0.2	0.8	0.0
	24	85	106	89	14.3	16.0	14.2
	21	114	67	88	19.2	10.1	14.1
	30	0	2	0	0.0	0.3	0.0
	20	68	47	52	11.4	7.1	8.3
	22	110	119	90	18.5	17.9	14.4
	25	50	79	93	8.4	11.9	14.9
	43.2	0	1	0	0.0	0.2	0.0
	19	34	43	45	5.7	6.5	7.2
	44.2S7.2	0	1	0	0.0	0.2	0.0
	26	16	19	47	2.7	2.9	7.5
	28S4	0	6	1	0.0	0.9	0.2
	24S7	0	1	0	0.0	0.2	0.0
	27S4	0	12	0	0.0	1.8	0.0
	18.2	0	8	0	0.0	1.2	0.0
	18	13	4	6	2.2	0.6	1.0
	44.2S7.2S4	0	1	0	0.0	0.2	0.0
	19.2	0	4	0	0.0	0.6	0.0
	44.2S7S4	0	1	0	0.0	0.2	0.0
	16.1	0	1	0	0.0	0.2	0.0
	20.2	0	2	0	0.0	0.3	0.0
	46.2S6.2S8	0	1	0	0.0	0.2	0.0
	29	0	2	1	0.0	0.3	0.2
	24S4	0	1	0	0.0	0.2	0.0
	28S4.2	0	1	0	0.0	0.2	0.0
	47.2S8.2S6	0	1	0	0.0	0.2	0.0
	26S4	1	4	0	0.2	0.6	0.0
	32.2	0	1	0	0.0	0.2	0.0
	17	0	1	0	0.0	0.2	0.0
	24.2	0	1	1	0.0	0.2	0.2
	17.2	0	1	0	0.0	0.2	0.0
	30.2	0	1	0	0.0	0.2	0.0
	23.2	5	1	0	0.8	0.2	0.0
	22.2	7	0	2	1.2	0.0	0.3
	25S12	2	0	1	0.3	0.0	0.2
	27	4	0	12	0.7	0.0	1.9
	21.2	2	0	3	0.3	0.0	0.5
	26S5	1	0	0	0.2	0.0	0.0
	28	0	0	5	0.0	0.0	0.8
	23S5	0	0	3	0.0	0.0	0.5
	25.3	0	0	1	0.0	0.0	0.2
	15	0	0	1	0.0	0.0	0.2
	25.2S4	0	0	1	0.0	0.0	0.2
	23.2S4	0	0	1	0.0	0.0	0.2
THO1	9	82	95	72	13.8	14.3	11.5
	7	123	293	230	20.7	44.1	36.7
	9.3	191	62	123	32.2	9.3	19.6
	8	55	126	44	9.3	19.0	7.0
	6	138	86	155	23.2	13.0	24.8
	10	2	2	2	0.3	0.3	0.3
	5	3	0	0	0.5	0.0	0.0

Polymorphism key:

Code	Polymorphism
S1	G->A
S2	A->G
S3	C->T
S4	T->C
S5	C->G
S6	G->C
S7	T->G
S8	G->T
S9	A->T
S10	T->A
S11	A->C
S12	C->A

Each polymorphism is encoded according to the table to the left
Multiple polymorphisms are indicated by a decimal point and numeric suffix
Combinations of polymorphisms are sequentially concatenated

Examples:

18S2 = 18 (A->G)
18S2.2 = 18 (2A->2G)
18S2.2S11 = 18 (2A->2G + A->C)

In addition to the 95 samples from NIST run in the preliminary 11-locus Y-STR assay, 187 samples obtained from John Planz at UNTHSC comprising 74 African American, 58 Caucasian and 45 Hispanic samples were run in the 16-locus (8-well) Y-STR assay. Although at least one SNP was observed in 12 of 16 loci, only three loci appeared to have a substantial number of polymorphic alleles. Each of these also appeared to present a level of population bias in SNP frequency for the three populations surveyed (Figure 47).

Locus	Caucasian SNP %	African American SNP %	Hispanic SNP %
DYS385a/b	0.0	0.9	1.7
DYS389II-1	5.6	67.3	17.0
DYS390	6.7	4.7	6.9
DYS392	1.1	0.9	0.0
DYS393	3.4	0.0	1.1
DYS437	1.1	59.4	4.6
DYS438	1.1	0.0	3.4
DYS439	0.0	0.0	1.1
DYS448	0.0	1.4	0.0
DYS458	6.9	1.4	5.5
DYS635	37.3	73.0	29.6
Y-GATA-H4	0.0	1.4	0.0

187 samples with 16-locus assay

- 74 African American
- 58 Caucasian
- 45 Hispanic

95 samples with preliminary 11-locus assay

- 32 African American
- 31 Caucasian
- 32 Hispanic

Figure 47. Frequency of SNPs observed in Y-STR loci for 187 samples surveyed at 16 loci and 95 samples surveyed at 11 loci.

Specific Aim 4: Analysis of extended family samples

A panel of samples received from UNTHSC containing groups of two parents plus one or more offspring where the sample set is known to contain parent/offspring combinations having parent-to-offspring STR mutations (e.g., an allele 12 from a parent becomes an allele 11 in the offspring) were tested in the Ibis STR system. The samples came blinded without information about the mutations in question or the parent-offspring relationships (other than code numbers that indicated which parents and offspring belonged together). The samples were genotyped using a scaled-down panel of primer pairs containing only the primer pairs known to contain a high frequency of SNP polymorphisms, namely D13S317, D21S11, D3S1358, D5S818, D7S820, D8S1179 and vWA. The loci were surveyed in custom plates containing two triplex reactions and one single-plex (D21S11), allowing 32 samples per 96-well plate to be analyzed (30 samples plus one positive and one negative control).

Profiles for the seven most polymorphic loci have been registered for 312 samples grouped into 97 defined family groupings containing a mother, a presumed father, and one or more offspring (Table 35). Of these groupings, there were 17 groups (55 total samples) for which one of the presumed parents did not appear to be consistent with the offspring or no germline mutation from parent to offspring was demonstrated. The remaining 257 samples were grouped into 80 family groupings where one offspring had a demonstrated germline mutation in an allele from one of the seven loci surveyed. Of these, 17 profiles were from offspring in multi-child families either unrelated to one of the parents or not containing a germline mutation in one of the seven loci, leaving 80 mother-father-offspring triplets with a verified germline mutation in one of the seven loci (Table 35, rows highlighted in light green).

Data shown in Table 35 clearly demonstrate that the polymorphisms observed in the STR alleles at the seven loci surveyed are faithfully transmitted from parent to

offspring and are not an artifact of the methodology used to assay them. We have not yet seen a demonstrable case of a two parent-offspring trio that suggests that an allele from a parent gained or lost a SNP polymorphism between a parent and a child. This makes sense, as the expected rate of base substitution mutation is much lower than that of replication slippage mutation in a repetitive element that leads to length polymorphism differences). For example, point mutation rates in genes of humans have been estimated at about $2x10^{-8}$ per nucleotide per generation, with hot-spot rates of $5x10^{-7}$ per nucleotide per generation or greater reportedly rare[1, 2]. Length-varying mutational rates in human STR loci (gain or loss of a repeat unit) have been estimated between ca. $5x10^{-4}$ per to $1x10^{-3}$ per generation[3, 4]. In informatics-based reports comparing predicted replication slippage rates to sequence polymorphism rates in STR loci, base substitution rates were predicted to be anywhere from 10 to 1000-fold lower than repeat slippage rates, which is consistent with independent predictions and measurements of the two types of events[5-7].

Each of the 80 trios highlighted in green in Table 35 have a verified case of length variation mutation between a parent and the offspring. It is interesting to note that in all 80 cases, the genotypes are consistent with a simple length variation without the necessity of hypothesizing any SNP polymorphism differences. For example, in group 15 (Table 35) there is an apparent mutation in D5S818 from allele 14 in the mother to allele 13 in the child, whereas in group 12 there is an apparent mutation in D5S818 from allele 14 (G→T) in the father to allele 13 (G→T) in the child. Note that both of these events would be seen as identical with conventional typing. With the mass spectrometry methodology, however, it is seen that there are actually two different sets of alleles (14 / 13 and 14 (G→T) / 13 (G→T)) and that a simple replication slippage-associated length mutation in 14 (G→T) leads to a 13 (G→T).

There are some rather interesting consequences of the increased discrimination of alleles afforded by the ability to detect polymorphisms within STR alleles when dealing with samples from related individuals. For example, if one examines the D5S818 genotypes that would results from standard typing for the three individuals of group 3, the genotypes would be: mother [11, 13], father [11, 13], child [12, 13]. It would therefore be considered possible that the mother contributed allele 13 and that the father contributed either an 11 or 13 that mutated to a 12, or alternatively that the father contributed allele 13 and that the mother contributed either an 11 or 13 that mutated to a 12. There would therefore be four distinct scenarios that could lead to the child's genotype. The mass spectrometry-based assay, however, produced the genotypes mother [11, 13], father [11, 13 (G→T)], child [12 (G→T), 13]. It is now straightforward to see that there is only one viable explanation for the path of mutation. The father's allele 13 (G→T) presumably mutated to a 12 (G→T) through replication slippage).

Another interesting case is group 61, sample UNTHSC0034-M0363C2, locus D8S1179. In this case, with conventional typing, the mother's genotype would be [14, 14], the father's would be [14, 15] and the child's would be [13, 14]. It would therefore be possible that, provided that these are the true parents (it could be imagined that this

could be a paternity case) the allele 13 could have come from the allele 14 of either the (known) mother or (assumed) father. With the mass spectrometry-based assay, the genotypes are mother [14, 14 (A→G)], father [14 (A→G), 15 (A→G)], and child [13, 14 (A→G)]. It is most plausible that the father actually contributed the allele 14 (A→G), requiring no hypothesis of a mutation in the father's germline, and the mother contributed her allele 14 that mutated to a 13 in the child. Figure 48 shows the data for these D8S1179 genotypes.

Table 35. Seven-locus profiles for members of 97 family groupings. Members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Locus	Change
1	UNTHSC0031-M0188A1	9, 12	30 (A->G), 31	17, 18	11, 11	10, 12 (T->A)	13, 13 (A->G)	16, 16		
1	UNTHSC0031-M0188B1	12, 12	29, 31.2	18, 18	11, 12 (G->T)	8, 12	13, 13 (A->G)	18, 19		
1	UNTHSC0031-M0188C1	12, 12	31, 31.2	17, 18	11, 11	10, 12	13, 13	16, 20	vWA	19->20
2	UNTHSC0031-M0033A1	11 (A->T), 12 (A->T)	31, 31	16 (G->A), 17	12, 14	10, 10	13 (A->G), 14 (A->G)	17, 18		
2	UNTHSC0031-M0033B1	11, 13 (A->T)	29 (G->A), 34.2	15 (2G->2A), 17	12, 13	8, 8	8, 14 (A->G)	16, 19		
2	UNTHSC0031-M0033C1	12 (A->T), 13 (A->T)	29 (G->A), 30 (A->G)	15 (2G->2A), 16 (G->A)	12, 12	8, 10	14 (A->G), 14 (A->G)	16, 17	D21S11	31->30
3	UNTHSC0031-M0096A1	12, 12	29, 30 (A->G)	16 (2G->2A), 16 (2G->2A)	11, 13	9, 11	13 (A->G), 13 (A->G)	16, 18		
3	UNTHSC0031-M0096B1	11, 12	27, 32.2	15 (G->A), 17	11, 13 (G->T)	10, 11 (T->A)	12 (A->G), 16 (A->G)	16 (G->A), 18		
3	UNTHSC0031-M0096C1	11, 12	29, 32.2	16 (2G->2A), 17	12 (G->T), 13	11, 11 (T->A)	13 (A->G), 16 (A->G)	16, 18	D5S818	13 (G->T)-> 12(G->T)
4	UNTHSC0031-M0073A1	9, 11	28, 31.2	15 (G->A), 16 (G->A)	11, 11	12, 12 (T->A)	10, 14 (A->G)	14 (A->G + 2T->2C), 19		
4	UNTHSC0031-M0073B1	12, 12	29 (G->A), 31 (G->A)	15 (G->A), 18	9 (G->T), 13	8, 13 (T->A)	10, 12	16, 17		
4	UNTHSC0031-M0073C1	11, 12	31 (G->A), 31.2	15 (G->A), 16 (G->A)	11, 13	12 (T->A), 12 (T->A)	12, 14 (A->G)	14 (A->G + 2T->2C), 16	D7S820	13 (T->A)-> 12 (T->A)
5	UNTHSC0031-M0072A1	12 (A->T), 13 (A->T)	28, 31	14 (G->A), 17	11, 11	11, 12	13, 14	14 (A->G + 2T->2C), 16		
5	UNTHSC0031-M0072B1	8, 9	30.2 (G->A), 31 (G->A)	15 (G->A), 16	11, 13	10, 12	10, 10	14 (A->G + 2T->2C), 15 (G->A)		
5	UNTHSC0031-M0072C1	8, 13 (A->T)	30.2 (G->A), 31	14 (G->A), 15 (G->A)	11, 11	11, 11	10, 13	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	D7S820	12->11 or 10->11
6	UNTHSC0031-M0143A1	8, 12	30 (A->G), 31 (G->A)	15 (G->A), 15 (G->A)	11 (G->T), 13 (G->T)	8, 10	13 (A->G), 15 (A->G)	17, 17		
6	UNTHSC0031-M0143B1	8, 13	29, 30 (A->G)	16 (2G->2A), 17 (G->A)	12, 12	8, 15	12, 14	17, 18		
6	UNTHSC0031-M0143C1	12, 13	30 (A->G), 31 (G->A)	15 (G->A), 16 (2G->2A)	11 (G->T), 12	8, 10	13, 15 (A->G)	17, 17	D8S1179	14->13 or 12->13
7	UNTHSC0031-M0296A1	12, 14	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 12 (G->T)	8, 8	12, 14 (A->G)	16, 20		
7	UNTHSC0031-M0296B1	12, 13	29, 32.2	16 (2G->2A), 16 (2G->2A)	11, 14 (G->T)	11, 11	14 (A->G), 16 (2A->2G)	17, 18		
7	UNTHSC0031-M0296C1	12, 14	30 (A->G), 32.2	15 (G->A), 16 (2G->2A)	11, 11	8, 11	14 (A->G), 17 (2A->2G)	18, 20	D8S1179	16 (2A->2G)-> 17 (2A->2G)
8	UNTHSC0031-M0292A1	12, 12 (A->T)	31.2 (G->A), 32.2	15 (G->A), 15 (G->A)	7, 12	11, 12	13, 13	17, 18		
8	UNTHSC0031-M0292B1	12, 12 (A->T)	29, 29	15 (G->A), 18 (G->C)	11, 12	10, 11	10, 14 (A->G)	14 (A->G + 2T->2C), 17		
8	UNTHSC0031-M0292C1	12, 12 (A->T)	29, 31.2 (G->A)	15 (G->A), 18 (G->C)	7, 12	11, 11	13, 14 (A->G)	17, 18		
9	UNTHSC0031-M0294A1	8, 11 (A->T)	29 (G->A), 30 (A->G)	15 (2G->2A), 16 (G->A)	10 (G->T), 11	10, 11	13, 13 (A->G)	17, 18		
9	UNTHSC0031-M0294B1	10 (A->T), 11	30, 30.2 (G->A)	16 (G->A), 17	12, 12	11, 11	12 (A->G), 13	17, 18		
9	UNTHSC0031-M0294C1	10 (A->T), 11 (A->T)	29 (G->A), 30	15 (2G->2A), 17	11, 12	11, 11	12 (A->G), 13	17, 17		
10	UNTHSC0031-M0293A1	12, 12 (A->T)	29, 31	15 (G->A), 16	11, 11	8, 11 (T->A)	13 (A->G), 14 (A->G)	16, 20		
10	UNTHSC0031-M0293B1	10, 11 (A->T)	28, 29 (G->A)	15 (G->A), 16 (G->A)	11, 12	11, 11	13 (A->G), 13 (A->G)	16, 17		
10	UNTHSC0031-M0293C1	11 (A->T), 12 (A->T)	29 (G->A), 31	15 (G->A), 15 (G->A)	11, 12	8, 11	13 (A->G), 13 (A->G)	16, 16		
11	UNTHSC0031-M0295A1	11, 11 (A->T)	30 (A->G), 30 (A->G)	16 (G->A), 18	10, 13 (G->C)	10, 12 (T->A)	10, 14 (A->G)	16, 19		
11	UNTHSC0031-M0295B1	11 (A->T), 12	27, 29	15 (G->A), 16 (2G->2A)	12 (G->T), 14	10, 12	13, 16 (A->G)	15, 16		
11	UNTHSC0031-M0295C1	11 (A->T), 11 (A->T)	27, 30 (A->G)	16 (2G->2A), 16 (G->A)	10, 12 (G->T)	10, 12	13, 14 (A->G)	16, 16		
12	UNTHSC0031-M0099A1	12, 13	31, 33.2	15 (G->A), 17 (G->A)	11, 12	11, 12	13, 14 (A->G)	15, 16 (G->A)		
12	UNTHSC0031-M0099B1	8, 9	25 (3A->3G), 30 (A->G)	15 (G->A), 18	12, 14 (G->T)	11, 12	12, 15 (A->G)	15 (G->A), 17		
12	UNTHSC0031-M0099C1	8, 13	25 (3A->3G), 31	15 (G->A), 18	11, 13 (G->T)	11, 11	13, 15 (A->G)	15, 15 (G->A)	D5S818	14 (G->T)-> 13 (G->T)
13	UNTHSC0032-M0311A1	11 (A->T), 12 (A->T)	29, 29	15 (2G->2A), 16 (2G->2A)	13, 13 (G->T)	8, 10	12, 12 (A->G)	15 (G->A), 16		
13	UNTHSC0032-M0311B1	12, 13 (A->T)	29 (A->G), 31	17 (G->A), 18	11, 12 (G->T)	9, 10	13 (A->G), 14 (A->G)	11, 14 (A->G + 2T->2C)		
13	UNTHSC0032-M0311C1	12 (A->T), 13 (A->T)	29, 32 (A->G)	15 (2G->2A), 17 (G->A)	11, 13	8, 9	12, 14 (A->G)	14 (A->G + 2T->2C), 15 (G->A)	D21S11	31->32
14	UNTHSC0032-M0313A1	12, 12 (A->T)	29, 30 (A->G)	15 (2G->2A), 16	12, 13 (G->C)	11, 12	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17 (G->A)		
14	UNTHSC0032-M0313B1	13, 13 (A->T)	28, 32.2	14 (G->A), 19 (G->A)	10, 12 (G->T)	8, 12	15 (2A->2G), 15 (A->G)	15 (G->A), 18 (G->A)		
14	UNTHSC0032-M0313C1	12 (A->T), 13 (A->T)	29, 32.2	14 (G->A), 16	12 (G->T), 13 (G->C)	8, 12	13 (A->G), 15 (2A->2G)	17 (G->A), 17 (G->A)	vWA	18 (G->A)->17 (G >A)
15	UNTHSC0032-M0315A1	10, 12	31.2, 32.2	14 (G->A), 18	11, 14	8, 11	10, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
15	UNTHSC0032-M0315B1	8, 9	32.2, 33.2	16 (2G->2A), 18	11, 13	10, 12	10, 13 (A->G)	15 (G->A), 16		
15	UNTHSC0032-M0315C1	8, 12	31.2, 32.2	14 (G->A), 18	13, 13	11, 12	10, 10	14 (A->G + 2T->2C), 15 (G->A)	D5S818	14->13
16	UNTHSC0032-M0316A1	11 (A->T), 12	30.2 (G->A), 31.2	16, 18	11, 12	10, 10 (T->A)	11, 12	16 (G->A), 19		
16	UNTHSC0032-M0316B1	10, 13	29, 34.2	14 (G->A), 15 (G->A)	12 (G->T), 13	8, 12	9, 13 (A->G)	15 (G->A), 17		
16	UNTHSC0032-M0316C1	11 (A->T), 12	29, 31.2	15 (G->A), 16	11, 13	10, 12	9, 12	15 (G->A), 16 (G->A)	D13S317	13->12
17	UNTHSC0032-M0317A1	11, 14	27 (A->G), 28	15 (G->A), 16 (G->A)	12 (G->T), 13	10, 11	13 (A->G), 14 (A->G)	15, 20 (A->G)		
17	UNTHSC0032-M0317C1	12, 14	28, 28	14 (G->A), 15 (G->A)	12, 13	11, 11	13 (A->G), 13 (A->G)	18, 20 (A->G)	vWA	19->18
17	UNTHSC0036-M0317B1	11, 12	28, 32 (A->G)	14 (G->A), 16 (G->A)	12, 12	10, 11	13 (A->G), 14 (A->G)	16, 19		
18	UNTHSC0032-M0318B1	12, 12	30, 32.2	16 (G->A), 16 (G->A)	11, 12	10, 11	10, 10	17, 20		
18	UNTHSC0032-M0318C1	10 (A->T), 12	30, 31	15 (G->A), 16 (G->A)	11, 11	10, 11	10, 10	17, 17	D21S11	30->31
18	UNTHSC0036-M0318A1	10 (A->T), 11 (A->T)	30 (A->G), 32 (A->G)	15 (G->A), 17	11, 11	11, 11	10, 12	16, 17		
19	UNTHSC0032-M0319A1	12 (A->T), 13	30 (A->G), 32.2	16 (G->A), 16 (G->A)	12 (G->T), 13	11, 11	10, 15 (A->G)	17, 18		
19	UNTHSC0032-M0319B1	9, 12 (A->T)	28, 31.2	16 (G->A), 16 (G->A)	9 (G->T), 12	9, 11	10, 11	17, 18		
19	UNTHSC0032-M0319C1	12 (A->T), 13	31.2, 32.2	16 (G->A), 16 (G->A)	9 (G->T), 12 (G->T)	11, 11	10, 15 (A->G)	16, 17	vWA	17->16
20	UNTHSC0032-M0320A1	12, 14	27 (A->G), 30 (A->G)	17 (G->A), 17 (G->A)	12, 13 (G->C)	10, 13	12 (A->G), 17 (2A->2G)	16, 16 (G->A)		
20	UNTHSC0032-M0320B1	11 (A->T), 12	28, 30.2	15, 15 (2G->2A)	9 (G->T), 12 (G->T)	8, 11	13 (A->G), 14 (A->G)	15 (G->A), 17		
20	UNTHSC0032-M0320C1	12, 14	27 (A->G), 28	16, 17 (G->A)	9 (G->T), 13 (G->C)	8, 13	12 (A->G), 14 (A->G)	15 (G->A), 16	D3S1358	15->16
21	UNTHSC0032-M0321A1	9, 12	30, 30 (A->G)	15 (G->A), 15 (G->A)	12 (G->T), 13	10, 11	13 (A->G), 13 (A->G)	16, 16		
21	UNTHSC0032-M0321B1	11, 11 (A->T)	27 (A->G), 30 (A->G)	16, 16 (2G->2A)	11, 13	8, 11	14 (A->G), 15 (A->G)	13 (C->T), 17		
21	UNTHSC0032-M0321C1	9, 11	30, 30 (A->G)	15 (2G->2A), 15 (G->A)	13, 13	10, 11	13 (A->G), 15 (A->G)	13 (C->T), 16	D3S1358	16 (2G->2A)-> 15(2G->2A)
21	UNTHSC0032-M0321C2	9, 11	27 (A->G), 30 (A->G)	15 (G->A), 16 (2G->2A)	11, 13	10, 11	13 (A->G), 14 (A->G)	13 (C->T), 16		
21	UNTHSC0032-M0321C3	9, 11 (A->T)	27 (A->G), 30	15 (G->A), 16 (2G->2A)	11, 12 (G->T)	11, 11	13 (A->G), 15 (A->G)	16, 17		
22	UNTHSC0032-M0322A1	10, 13	30 (A->G), 32.2	18, 18	9 (G->T), 11	9, 11	13 (A->G), 13 (A->G)	16, 17		
22	UNTHSC0032-M0322B1	9, 10	30, 31	18, 18	13, 14	10, 11	14 (A->G), 15 (A->G)	16, 17		
22	UNTHSC0032-M0322C1	9, 10	30 (A->G), 31	18, 18	9 (G->T), 14	11, 11	13 (A->G), 16 (A->G)	17, 17	D8S1179	15 (A->G)-> 16 (A->G)
23	UNTHSC0032-M0323A1	11, 12	30, 32.2	16 (G->A), 18	12, 13 (G->T)	11, 11	13, 13 (A->G)	15 (G->A), 16		
23	UNTHSC0032-M0323B1	11, 12 (A->T)	30, 32.2	15 (G->A), 16	10 (G->T), 12	9, 11	13, 15 (A->G)	17, 18		
23	UNTHSC0032-M0323C1	11, 12	32.2, 32.2	16, 18	10 (G->T), 12	11, 11	12, 13	16, 17	D8S1179	13->12
24	UNTHSC0032-M0325B1	12, 12	27 (A->G), 29.2 (A->G)	15 (G->A), 18	11, 12	10 (T->A), 12	10, 13	18, 18		
24	UNTHSC0032-M0325C1	12, 12 (A->T)	29.2 (A->G), 33.2	16 (G->A), 18	11, 11	10, 12	11, 14	15 (G->A), 18	D8S1179	13->14
24	UNTHSC0036-M0325A1	12, 12 (A->T)	29 (G->A), 33.2	16 (2G->2A), 16 (G->A)	11, 11	10, 14	11, 11	15 (G->A), 18 (G->A)		
25	UNTHSC0032-M0328A1	12, 12	28, 29	16 (2G->2A), 16 (G->A)	11, 11	10, 10	12, 15 (A->G)	15, 19 (2A->2G)		
25	UNTHSC0032-M0328B1	11, 11 (A->T)	28, 31	14 (G->A), 17	12 (G->T), 13	9, 11 (T->A)	13, 14 (A->G)	15, 20 (A->G)		
25	UNTHSC0032-M0328C1	11 (A->T), 12	28, 29	16 (2G->2A), 17	11, 13	10, 10 (T->A)	13, 15 (A->G)	15, 20 (A->G)	D7S820	11 (T->A)-> 10 (T->A)
26	UNTHSC0032-M0300A1	8, 10 (A->T)	29, 32 (A->G)	15 (G->A), 16 (G->A)	12, 12 (G->T)	11, 11 (T->A)	13 (A->G), 14 (A->G)	19, 20		
26	UNTHSC0032-M0300B1	9, 11	28, 30 (A->G)	15 (G->A), 16 (G->A)	9 (G->T), 12	9, 10	10, 13 (A->G)	17, 17		
26	UNTHSC0036-M0300C1	8, 11	30 (A->G), 32 (A->G)	15 (G->A), 16 (G->A)	12, 12 (G->T)	9, 11	13 (A->G), 14 (A->G)	16, 20	vWA	17->16
27	UNTHSC0032-M0301A1	8, 12	31.2, 32.2	15, 18	10, 11	10, 12	13 (A->G), 13 (A->G)	15 (G->A), 16 (G->A)		
27	UNTHSC0032-M0301B1	11, 14	29, 33.2	14 (2G->2A), 18 (G->A)	10, 10	10, 10	14 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 17 (G->A)		
27	UNTHSC0032-M0301C1	12, 14	29, 31.2	14 (2G->2A), 15	11, 11	10, 10	13 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 15 (G->A)	D5S818	10->11
28	UNTHSC0032-M0302A1	10 (A->T), 11	28, 30 (A->G)	14 (G->A), 17	10, 12	12, 12 (T->A)	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 16		
28	UNTHSC0032-M0302B1	12, 12	30 (A->G), 31.2	14 (G->A), 16 (G->A)	11, 12	12, 12	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 17		
28	UNTHSC0032-M0302C1	11, 12	30 (A->G), 30 (A->G)	14 (G->A), 14 (G->A)	10, 12	11, 12	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	D7S820	12->11
29	UNTHSC0032-M0306A1	9, 12	30 (A->G), 32.2	15 (G->A), 17	11, 12	9, 13	10, 14 (A->G)	17, 21		
29	UNTHSC0032-M0306B1	9, 11 (A->T)	29 (G->A), 31	16, 18	10 (G->T), 12 (G->T)	9, 10 (T->A)	14 (A->G), 16 (A->G)	15 (G->A), 18		
29	UNTHSC0032-M0306C1	9, 9	30 (A->G), 31	15 (G->A), 18	12, 12 (G->T)	9, 13	10, 14 (A->G)	18, 21		
30	UNTHSC0032-M0307A1	11 (A->T), 12	30 (A->G), 32.2	15 (G->A), 15 (G->A)	11, 12	10, 11	10, 13 (A->G)	16, 18		
30	UNTHSC0032-M0307C1	11 (A->T), 12 (A->T)	30, 32.2	15 (G->A), 16 (G->A)	11, 11	11, 11	10, 14 (A->G)	17 (G->A), 18	D8S1179	15 (A->G)-> 14 (A->G) or 13 (A->G)-> 14 (A->G)
30	UNTHSC0036-M0307B1	12 (A->T), 13	30, 31 (G->A)	16 (G->A), 16 (G->A)	11, 13	10, 11	13 (A->G), 15 (A->G)	17 (G->A), 18		
31	UNTHSC0032-M0308A1	12, 12	29 (G->A), 32.2	17, 17 (G->A)	11, 12	7, 12 (T->A)	13 (A->G), 14	14 (A->G + 2T->2C), 18		
31	UNTHSC0032-M0308B1	8, 13	32.2, 33.2	16, 16 (G->A)	12, 12	10, 10	13 (A->G), 15 (A->G)	16, 17		
31	UNTHSC0032-M0308C1	12, 13	29 (G->A), 33.2	16, 17	12, 12	10, 12 (T->A)	13 (A->G), 16 (A->G)	16, 18	D8S1179	15 (A->G)-> 16 (A->G)
32	UNTHSC0032-M0309A1	11, 13	28, 30	15 (G->A), 16	11, 12	10, 11	13 (A->G), 14 (A->G)	15, 15 (G->A)		
32	UNTHSC0032-M0309B1	12 (A->T), 14	28, 30	14 (G->A), 19	12, 14	8, 8	11, 13 (A->G)	15, 17		
32	UNTHSC0032-M0309C1	12 (A->T), 13	30, 30	14 (G->A), 15 (G->A)	12, 15	8, 11	11, 13 (A->G)	15 (G->A), 17	D5S818	14->15
33	UNTHSC0032-M0310A1	12, 13	28, 30	15 (2G->2A), 16 (G->A)	11, 11	11, 12	10, 14	15 (G->A), 17		
33	UNTHSC0032-M0310B1	11, 13	30 (A->G), 33.2	14 (G->A), 15 (G->A)	11, 12	9, 9	12, 16 (A->G)	18, 18		
33	UNTHSC0032-M0310C1	12, 13	28, 33.2	15 (2G->2A), 15 (G->A)	11, 12	9, 12	10, 16 (A->G)	17, 17	vWA	18->17

Table 35, continued. Seven-locus profiles for members of 97 family groupings. Members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Locus	Change
34	UNTHSC0032-M0329B1	11 (A->T), 12	28, 29	16 (2G->2A), 17 (G->A)	8 (G->T), 12 (G->T)	10, 11	13, 16 (A->G)	14 (T->C), 16		
34	UNTHSC0032-M0329C1	11, 13	28, 31.2 (G->A)	15 (G->A), 15 (G->A)	7, 11	8, 10	12 (A->G), 15 (A->G)	15, 18		
34	UNTHSC0036-M0329A1	11, 11 (A->T)	30, 31.2 (G->A)	15 (G->A), 15 (G->A)	7, 13 (G->T)	8, 13	14 (A->G), 15 (A->G)	15, 17		
34	UNTHSC0036-M0329C2	11, 13	28, 31.2 (G->A)	15 (G->A), 15 (G->A)	12, 13 (G->T)	8, 11	12 (A->G), 15 (A->G)	17, 18		
35	UNTHSC0032-M0330A1	10, 12	28, 31.2	15 (G->A), 17	13, 13 (G->T)	8, 10	13 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 18		
35	UNTHSC0032-M0330B1	12, 12 (A->T)	27, 28	15 (2G->2A), 17 (G->A)	12, 13 (G->T)	10, 10	13 (A->G), 14 (A->G)	16, 18		
35	UNTHSC0033-M0330C1	12, 12 (A->T)	28, 28	15 (2G->2A), 17	12, 13	10, 11	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 16	D7S820	10->11
36	UNTHSC0033-M0331A1	12, 12	28, 28	14 (2G->2A), 16 (2G->2A)	12, 12 (G->T)	8, 8	14 (A->G), 16 (A->G)	18 (2A->2G), 19		
36	UNTHSC0033-M0331C1	12, 12	28, 32 (A->G)	14 (2G->2A), 16	12, 13	8, 8	15 (A->G), 16 (A->G)	18, 19	D5S818	12->13
36	UNTHSC0036-M0331B1	12, 13	29, 32 (A->G)	15 (2G->2A), 16	8 (G->T), 12	8, 8	13 (A->G), 15 (A->G)	18, 19		
37	UNTHSC0033-M0332A1	11, 13	31.2, 33.2	15 (G->A), 16 (G->A)	9 (G->T), 11 (G->T)	10, 10 (T->A)	12 (A->G), 14	15, 16		
37	UNTHSC0033-M0332B1	9, 12 (A->T)	28, 30	15 (G->A), 16 (G->A)	12, 13	8, 10	12 (A->G), 14	16, 16		
37	UNTHSC0033-M0332C1	11, 12 (A->T)	28, 32.2	16 (G->A), 16 (G->A)	11 (G->T), 13	8, 10 (T->A)	12 (A->G), 12 (A->G)	15, 16	D21S11	31.2->32.2 or 33.2->32.2
38	UNTHSC0033-M0333A1	8, 11 (A->T)	30 (A->G), 30 (A->G)	14 (G->A), 16	11, 11	9, 11	10, 12	14 (A->G + 2T->2C), 16		
38	UNTHSC0033-M0333B1	8, 11	31, 31.2	14 (G->A), 16 (G->A)	13 (G->T), 13 (G->T)	11, 11	no data	17, 17		
38	UNTHSC0033-M0333C1	8, 11 (A->T)	30 (A->G), 31	16, 16 (G->A)	12, 13 (G->T)	9, 11	12, 12	14 (A->G + 2T->2C), 15	D5S818	11->12
39	UNTHSC0033-M0335A1	12, 12 (A->T)	28, 31.2	15 (G->A), 18	8 (G->T), 12	8, 11 (T->A)	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 15		
39	UNTHSC0033-M0335B1	11, 12 (A->T)	32 (A->G), 32.2	15 (G->A), 17	11, 13 (G->C)	9, 9	14 (A->G), 14 (A->G)	15, 18 (G->A)		
39	UNTHSC0033-M0335C1	12 (A->T), 12 (A->T)	31.2, 32.2	15 (G->A), 17	8 (G->T), 13 (G->C)	11 (T->A), 11 (T->A)	13 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17 (G->A)	vWA	18 (G->A)-> 17 (G->A)
40	UNTHSC0033-M0336A1	8, 12 (A->T)	29, 31	14 (G->A), 18	7, 12	11, 13	14 (A->G), 14 (A->G)	16, 17		
40	UNTHSC0033-M0336B1	9, 12 (A->T)	30 (A->G), 32.2	17, 17	11, 12	10, 12	15 (A->G), 15 (A->G)	16, 18		
40	UNTHSC0033-M0336C1	12 (A->T), 12 (A->T)	29, 32.2	14 (G->A), 17	7, 12	10, 11	14 (A->G), 15 (A->G)	14 (A->G + 2T->2C), 17		
41	UNTHSC0033-M0339A1	11 (A->T), 13	30 (A->G), 31.2	16 (G->A), 18	10, 11	11 (T->A), 12	8, 10	16, 17		
41	UNTHSC0033-M0339B1	11, 12 (A->T)	29 (A->G), 30 (A->G)	17 (G->A), 17 (G->A)	11, 13	11, 12 (T->A)	10, 13 (A->G)	14 (A->G + 2T->2C), 20		
41	UNTHSC0033-M0339C1	11 (A->T), 12 (A->T)	29 (A->G), 30 (A->G)	16 (G->A), 17 (G->A)	10, 13	11, 12	8, 13 (A->G)	16, 19	vWA	20->19
42	UNTHSC0033-M0341A1	11 (A->T), 12	29, 31 (G->A)	15 (2G->2A), 17	11, 12	10, 11	13 (A->G), 16 (A->G)	16, 18		
42	UNTHSC0033-M0341B1	11, 11 (A->T)	27, 29	15 (2G->2A), 16 (G->A)	12, 13 (G->C)	11, 11	14 (A->G), 14 (A->G)	15 (G->A), 19 (G->A)		
42	UNTHSC0033-M0341C1	11, 11 (A->T)	27, 29	15 (2G->2A), 17	11, 13 (G->C)	11, 11	13 (A->G), 14 (A->G)	18, 18 (G->A)	vWA	19 (G->A)-> 18 (G->A)
43	UNTHSC0033-M0342A1	8, 12 (A->T)	29, 31.2	13 (2G->2A), 16 (2G->2A)	11, 13	11, 11	14 (A->G), 14 (A->G)	15 (G->A), 19		
43	UNTHSC0033-M0342B1	11, 14	28, 31.2	14 (G->A), 16 (G->A)	13 (G->T), 14	10, 10	14 (A->G), 14 (A->G)	16, 19		
43	UNTHSC0033-M0342C1	8, 14	28, 29	16 (2G->2A), 16 (G->A)	11, 13 (G->T)	10, 11	13 (A->G), 14 (A->G)	15 (G->A), 16	D8S1179	14 (A->G)-> 13 (A->G)
44	UNTHSC0033-M0343A1	8, 12 (A->T)	31, 32.2	15 (G->A), 17 (A->G)	12, 12	10, 10	11, 13	17, 17 (G->A)		
44	UNTHSC0033-M0343B1	11, 14	29, 31	14 (G->A), 16 (G->A)	11, 12	9, 12 (T->A)	14 (A->G), 14 (A->G)	14 (A->G + 2T->2C), 17		
44	UNTHSC0033-M0343C1	8, 9	29, 30 (A->G)	17 (A->G), 17 (G->A)	12, 13	10, 11	11, 13	14 (A->G + 2T->2C), 17		
45	UNTHSC0033-M0344A1	9, 9	34.2, 34.2	15 (G->A), 15 (G->A)	11, 11 (G->T)	11, 12	13 (A->G), 14 (A->G)	14 (T->C), 17		
45	UNTHSC0033-M0344B1	9, 11 (A->T)	31, 31.2	15 (G->A), 16 (2G->2A)	11, 12	10, 12	13 (A->G), 14 (A->G)	16, 16 (G->A)		
45	UNTHSC0033-M0344C2	9, 11 (A->T)	31, 31	15 (G->A), 15 (G->A)	11, 11	10, 12	13 (A->G), 13 (A->G)	14 (T->C), 16		
46	UNTHSC0033-M0345A1	12, 12	28, 31	16 (2G->2A), 16 (2G->2A)	13, 13	9, 10	11, 16 (A->G)	14 (A->G + 2T->2C), 15 (G->A)		
46	UNTHSC0033-M0345B1	11 (A->T), 12	27 (A->G), 30	15 (G->A), 16 (G->A)	11, 12 (G->T)	9, 11	14 (A->G), 14 (A->G)	19, 19		
46	UNTHSC0033-M0345C1	12, 12	30, 31	15 (G->A), 16 (2G->2A)	11, 13	9, 11	14 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 20	vWA	19->20
47	UNTHSC0033-M0346A1	13, 14	28, 30 (A->G)	15 (G->A), 17 (G->A)	11, 11	10, 12	14 (A->G), 15 (A->G)	16, 17		
47	UNTHSC0033-M0346B1	10, 13	29, 30 (A->G)	15 (G->A), 17	11, 11	10, 13	13, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
47	UNTHSC0033-M0346C1	12, 13	30 (A->G), 30 (A->G)	17, 17 (G->A)	11, 11	12, 13	13, 14 (A->G)	14 (A->G + 2T->2C), 16	D13S317	13->12
48	UNTHSC0033-M0347A1	8, 12	29, 29	15 (G->A), 16	9 (G->T), 11 (G->T)	8, 9	12, 13 (A->G)	17 (G->A), 18		
48	UNTHSC0033-M0347C1	11 (A->T), 12	28, 29	15 (G->A), 15 (G->A)	11 (G->T), 12	9, 9	12, 16 (A->G)	18, 18 (G->A)	vWA	19 (G->A)-> 18 (G->A)
48	UNTHSC0036-M0347B1	11 (A->T), 12	28, 30 (A->G)	15 (G->A), 16 (G->A)	11, 12	8, 9	15 (A->G), 16 (A->G)	14 (A->G + 2T->2C), 19 (G->A)		
49	UNTHSC0033-M0348B1	12, 13 (A->T)	30 (A->G), 31	15, 15 (G->A)	13, 14 (G->T)	10, 10	14 (A->G), 14 (A->G)	15, 17 (G->A)		
49	UNTHSC0033-M0348C1	12, 12	30 (A->G), 31	15, 15 (G->A)	11, 13	8, 10	12, 14 (A->G)	17 (G->A), 17 (G->A)		
49	UNTHSC0036-M0348A1	12, 14	30 (A->G), 30 (A->G)	12 (2G->2A), 15 (G->A)	11, 11	8, 11	12, 13 (A->G)	16, 16		
50	UNTHSC0033-M0349A1	11, 14	30.2, 32.2	14 (2G->2A), 17	11, 12 (G->T)	7, 8	12 (A->G), 16 (2A->2G)	16, 17		
50	UNTHSC0033-M0349B1	10 (A->T), 12	29, 31	15, 15 (2G->2A)	11, 13 (G->C)	9, 11	14 (A->G), 16 (A->G)	18, 20		
50	UNTHSC0033-M0349C1	14, 14	29, 30.2	14 (2G->2A), 15 (2G->2A)	12 (G->T), 13 (G->C)	7, 9	14 (A->G), 17 (2A->2G)	16, 18	D8S1179	16 (2A->2G) -> 17 (2A->2G)
51	UNTHSC0033-M0350A1	8, 12	28 (A->G), 29	16 (G->A), 18	11, 12	11 (T->A), 12	12, 13 (A->G)	15 (G->A), 19		
51	UNTHSC0033-M0350B1	11, 11	28, 28	14 (G->A), 15 (2G->2A)	12 (G->T), 12 (G->T)	10, 11	12 (A->G), 14 (A->G)	16, 20		
51	UNTHSC0033-M0350C1	8, 11	28, 28 (A->G)	15 (2G->2A), 16 (G->A)	11, 12 (G->T)	11, 11 (T->A)	12, 12 (A->G)	19, 21	vWA	20->21
52	UNTHSC0033-M0351A1	11 (A->T), 11 (A->T)	30, 32.2	15 (G->A), 17	11, 12	9 (T->C), 13	11, 13	14 (A->G + 2T->2C), 17		
52	UNTHSC0033-M0351B1	11 (A->T), 11 (A->T)	28, 30 (A->G)	16 (G->A), 18	11, 12	9, 10 (T->A)	12, 15 (A->G)	18, 18		
52	UNTHSC0033-M0351C1	11 (A->T), 11 (A->T)	28, 30	16 (G->A), 17	11, 12	9, 13	11, 15 (A->G)	14 (A->G + 2T->2C), 17	vWA	18->17
53	UNTHSC0033-M0352A1	11, 14	30 (A->G), 31	15 (G->A), 16 (2G->2A)	11 (G->T), 12	10, 11	14 (A->G), 15 (A->G)	17, 17 (G->A)		
53	UNTHSC0033-M0352B1	11 (A->T), 12	28, 30 (A->G)	17, 18 (G->A)	12 (G->T), 14	8, 10	10, 14 (A->G)	15, 16		
53	UNTHSC0033-M0352C1	11, 11 (A->T)	30 (A->G), 31	16 (2G->2A), 18 (G->A)	12, 13	10, 11	10, 14 (A->G)	16, 17	D5S818	14->13
54	UNTHSC0033-M0353A1	10 (A->T), 12	28, 29	14 (G->A), 15 (G->A)	12, 12	9, 12	13 (A->G), 14 (A->G)	16, 17		
54	UNTHSC0033-M0353B1	9, 12 (A->T)	29 (G->A), 30 (A->G)	15 (G->A), 18	7, 13	8, 10	13 (A->G), 14 (A->G)	11, 19		
54	UNTHSC0033-M0353C1	10 (A->T), 12 (A->T)	28, 29 (G->A)	14 (G->A), 15 (G->A)	12, 13	8, 12	13 (A->G), 14 (A->G)	16, 20	vWA	19->20
55	UNTHSC0033-M0355A1	12, 12	30 (A->G), 34 (A->G)	15 (2G->2A), 17	11, 13	8, 8	13 (A->G), 15 (A->G)	17, 17		
55	UNTHSC0033-M0355B1	10 (A->T + G->A), 13	28, 32.2	15 (2G->2A), 16 (G->A)	12, 13 (G->C)	10, 11	13, 15 (A->G)	16, 19 (G->A)		
55	UNTHSC0036-M0355C1	12, 13	28, 30 (A->G)	16 (G->A), 17	11, 12	8, 10	12, 15 (A->G)	16, 17	D8S1179	13->12
56	UNTHSC0033-M0356A1	12, 13	29, 30	15 (G->A), 16 (G->A)	12, 12	10, 11	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)		
56	UNTHSC0033-M0356B1	12, 13	29 (G->A), 30 (A->G)	14 (G->A), 15 (G->A)	12, 12	11 (T->A), 13	13 (A->G), 15	14 (T->C), 16		
56	UNTHSC0033-M0356C1	12, 12	29, 29 (G->A)	14 (G->A), 16 (G->A)	12, 12	11, 13	13 (A->G), 14	14 (A->G + 2T->2C), 16	D8S1179	15->14
57	UNTHSC0033-M0357A1	9, 13	29, 30 (A->G)	15 (G->A), 16 (G->A)	11, 11	9, 11	14 (A->G), 14 (A->G)	16, 20		
57	UNTHSC0033-M0357B1	10 (A->T), 13	29, 30 (A->G)	15 (G->A), 16	11, 12	10, 10	13, 16 (A->G)	16, 17		
57	UNTHSC0033-M0357C1	13, 13	29, 30 (A->G)	15 (G->A), 16 (G->A)	11, 12	9, 10	14 (A->G), 15 (A->G)	16, 20	D8S1179	16 (A->G)-> 15 (A->G)
58	UNTHSC0033-M0358B1	11, 12	29, 33.2	15 (G->A), 16 (G->A)	11, 11	9, 10	14 (A->G), 14 (A->G)	16, 18		
58	UNTHSC0033-M0358C1	9, 11	30 (A->G), 33.2	15 (G->A), 15 (G->A)	7, 11	10, 10	13, 14 (A->G)	16, 17		
58	UNTHSC0036-M0358A1	9, 12	29, 33.2	14 (2G->2A), 15 (G->A)	11, 12	9, 11	13 (A->G), 14 (A->G)	18, 18		
59	UNTHSC0033-M0359A1	8, 12	30 (A->G), 30 (A->G)	14 (G->A), 16	10, 12 (G->T)	9, 12	13, 17 (A->G)	16, 18		
59	UNTHSC0033-M0359C1	8, 12 (A->T)	29 (G->A), 30 (A->G)	14 (G->A), 16 (2G->2A)	10, 11	9, 12	13, 14 (A->G)	17, 18		
59	UNTHSC0033-M0359C2	12, 12 (A->T)	30 (A->G), 30 (A->G)	16, 16 (2G->2A)	12 (G->T), 13	9, 9	14 (A->G), 17 (A->G)	16, 17	D5S818	12->13
59	UNTHSC0036-M0359B1	11 (A->T), 12 (A->T)	29 (G->A), 30 (A->G)	14 (G->A), 16 (2G->2A)	11, 12	9, 12	14 (A->G), 14 (A->G)	15 (G->A), 17		
60	UNTHSC0034-M0361A1	9, 11 (A->T)	30 (A->G), 32.2	14 (G->A), 16 (G->A)	7, 12	10, 11	14, 15 (A->G)	13 (G->A + C->T), 17		
60	UNTHSC0034-M0361B1	9, 11	30, 30 (A->G)	16 (G->A), 17 (G->A)	12 (G->T), 13	11, 11	12, 15 (A->G)	15 (G->A), 17		
60	UNTHSC0036-M0361C1	11, 11 (A->T)	30 (A->G), 32.2	16 (G->A), 16 (G->A)	12, 14	10, 11	12, 14	15 (G->A), 17	D5S818	13->14
61	UNTHSC0034-M0363A1	13, 14	29, 33.2	15 (G->A), 15 (G->A)	10, 10 (G->T)	10, 11	14, 14 (A->G)	16, 18		
61	UNTHSC0034-M0363B1	12, 14	31.2, 32.2	15 (G->A), 15 (G->A)	12, 13	10, 10	14 (A->G), 15 (A->G)	16, 17		
61	UNTHSC0034-M0363C1	9, 13	29, 29	15 (G->A), 16 (G->A)	10, 11	10, 11	10, 14	16, 18		
61	UNTHSC0034-M0363C2	12, 14	31.2, 33.2	15 (G->A), 15 (G->A)	10 (G->T), 12	10, 11	13, 14 (A->G)	17, 18	D8S1179	14->13
61	UNTHSC0034-M0363C3	12, 13	29, 31.2	15 (G->A), 15 (G->A)	10, 13	10, 10	14 (A->G), 14 (A->G)	17, 18		
62	UNTHSC0034-M0364B1	11, 14	29, 30 (A->G)	16 (G->A), 17	11, 12	8, 11	13 (A->G), 15 (A->G)	15 (G->A), 16		
62	UNTHSC0034-M0364C1	9, 13	28, 30 (A->G)	15 (G->A), 17	11, 11	8, 8	13, 13 (A->G)	16, 17	D13S317	14->13
62	UNTHSC0036-M0364A1	9, 9	28, 30	15 (2G->2A), 15 (G->A)	11, 11	8, 9	10, 13	16, 17		
63	UNTHSC0034-M0365A1	13, 14	29, 31.2	16 (G->A), 16 (G->A)	11 (G->T), 12 (G->T)	7, 11	10, 16 (A->G)	17, 19 (A->G)		
63	UNTHSC0034-M0365B1	11 (A->T), 14	28, 32.2	15 (G->A), 17 (G->A)	9 (G->T), 12	8, 11	13, 14 (A->G)	16, 16		
63	UNTHSC0034-M0365C1	14, 14	29, 32.2	16 (G->A), 17 (G->A)	9 (G->T), 12 (G->T)	8, 11	12, 16 (A->G)	16, 19 (A->G)	D8S1179	13->12
63	UNTHSC0034-M0365C2	11 (A->T), 14	29, 32.2	16 (G->A), 17 (G->A)	11 (G->T), 12	8, 11	10, 13	16, 17		
64	UNTHSC0034-M0368A1	13, 14	31.2, 33.2	14 (G->A), 15 (G->A)	7, 11	9, 11	11, 14 (A->G)	14 (A->G + 2T->2C), 17		
64	UNTHSC0034-M0368B1	12, 12 (A->T)	30 (A->G), 32 (A->G)	17, 18	12 (G->T), 13	11, 11	13, 13 (A->G)	16, 19		
64	UNTHSC0034-M0368C1	12, 13	30 (A->G), 31.2	15 (G->A), 17	7, 14	9, 11	11, 13	14 (A->G + 2T->2C), 16	D5S818	13->14
65	UNTHSC0034-M0370A1	12 (A->T), 13	no data	16 (G->A), 17 (G->A)	11, 12	11, 14	11, 13 (A->G)	17, 17		
65	UNTHSC0034-M0370B1	11 (A->T), 14	28 (G->A), 30	15 (G->A), 18	12 (G->T), 13	10, 10	12, 14 (A->G)	15 (G->A), 18		
65	UNTHSC0034-M0370C1	13, 14	29, 30	16 (G->A), 18	11, 12 (G->T)	10, 14	11, 14 (A->G)	17, 17	vWA	18->17
66	UNTHSC0034-M0371A1	9, 11 (A->T)	28, 30 (A->G)	15 (G->A), 18	11, 11	9, 13 (T->A)	15 (A->G), 15 (A->G)	17, 17		
66	UNTHSC0034-M0371B1	8, 9	28, 29	15 (2G->2A), 16	11, 12 (G->T)	9, 13 (T->A)	10, 13 (A->G)	17, 17		
66	UNTHSC0034-M0371C1	8, 11 (A->T)	28, 30 (A->G)	15 (2G->2A), 18	11, 11	9, 13 (T->A)	13 (A->G), 15 (A->G)	16, 17	vWA	17->16

Table 35, continued. Seven-locus profiles for members of 97 family groupings. Members of 80 mother-father-offspring trios containing a verified germline mutation are highlighted in light green.

Group	Sample	D13S317	D21S11	D3S1358	D5S818	D7S820	D8S1179	vWA	Mutated locus	From -> To
67	UNTHSC0034-M0372A1	12, 12 (A->T)	29, 30 (A->G)	14 (G->A), 17	8 (G->T), 13	11, 13	15 (A->G), 15 (A->G)	15, 15		
67	UNTHSC0034-M0372B1	11 (A->T), 12	27 (A->G), 30 (A->G)	14 (G->A), 19	11, 14	10, 13	14 (A->G), 14 (A->G)	17, 19		
67	UNTHSC0034-M0372C1	11 (A->T), 12	30 (A->G), 30 (A->G)	14 (G->A), 17	11, 13	10, 13	14 (A->G), 15 (A->G)	16, 19	vWA	15->16
68	UNTHSC0034-M0373A1	10, 12	29 (G->A), 30.2	15 (G->A), 16 (G->A)	11, 12	10, 10	14 (A->G), 14 (A->G)	15, 16		
68	UNTHSC0034-M0373B1	11 (A->T), 13	30 (A->G), 31.2	15 (G->A), 17	11, 11	11, 11 (T->A)	13, 13 (A->G)	14 (A->G + 2T->2C), 17		
68	UNTHSC0034-M0373C1	11 (A->T), 12	29 (G->A), 31.2	16 (G->A), 17	11, 11	10, 11	13, 14 (A->G)	16, 16	vWA	17->16
69	UNTHSC0034-M0374A1	12 (A->T), 12 (A->T)	28, 32.2	14 (G->A), 15 (G->A)	11, 12 (G->T)	8, 11	13, 13 (A->G)	16, 18		
69	UNTHSC0034-M0374B1	12, 12 (A->T)	30, 34.2	15 (G->A), 16 (G->A)	8 (G->T), 11	9 (T->A), 11	12, 13 (A->G)	16, 16		
69	UNTHSC0034-M0374C1	12 (A->T), 13 (A->T)	30, 32.2	15 (G->A), 16 (G->A)	11, 12 (G->T)	9 (T->A), 11	12, 13	16, 16	D13S317	12 (A->T)-> 13 (A->T)
70	UNTHSC0034-M0375A1	12 (A->T), 13	30 (A->G), 32.2	15, 18	12, 12	9, 10	12, 13	16 (G->A), 21 (2A->2G)		
70	UNTHSC0034-M0375B1	11, 12	29, 30 (A->G)	16, 16	11, 11	11, 12	12, 14 (A->G)	17, 17		
70	UNTHSC0034-M0375C1	11, 12	28, 28	15 (G->A), 16	11, 12 (G->T)	8, 11	12 (A->G), 16 (A->G)	16, 19 (A->G)		
71	UNTHSC0034-M0376A1	12 (A->T), 14	29, 29 (G->A)	16, 18	12, 13	11, 13	14 (A->G), 16 (2A->2G)	14 (2A->2G + 2T->2C), 14 (2A->2G + 2T->2C)		
71	UNTHSC0034-M0376B1	10, 13	28, 29 (G->A)	16 (G->A), 17 (G->A)	12, 12 (G->T)	11, 11	12, 14	15, 15		
71	UNTHSC0034-M0376C2	12 (A->T), 13	29 (G->A), 29 (G->A)	16, 17 (G->A)	12, 12 (G->T)	11, 11	14 (A->G), 15	13 (C->T), 15	D8S1179	14->15
71	UNTHSC0036-M0376C1	13, 14	28, 29 (G->A)	16, 16 (G->A)	12 (G->T), 13	11, 13	12, 14 (A->G)	14 (2A->2G + 2T->2C), 15		
72	UNTHSC0034-M0377B1	11 (A->T), 11 (A->T)	31, 31.2	16 (2G->2A), 17	12 (G->T), 13	8, 11	14 (A->G), 14 (A->G)	14 (T->C), 15		
72	UNTHSC0034-M0377C1	11 (A->T), 13	27, 31	14 (2G->2A), 16 (2G->2A)	13, 13 (G->T)	8, 11	12 (A->G), 15 (A->G)	13 (C->T), 15		
72	UNTHSC0036-M0377A1	11, 13	27, 28	14 (2G->2A), 15 (2G->2A)	11, 13 (G->T)	10, 11	15 (2A->2G), 15 (A->G)	13 (C->T), 14 (A->G + 2T->2C)		
73	UNTHSC0034-M0378A1	11 (A->T), 12	29 (A->G), 30.2	16, 16 (G->A)	13, 13 (G->T)	9, 10	10, 13 (A->G)	14 (A->G + 2T->2C), 16 (G->A)		
73	UNTHSC0034-M0378B1	12 (A->T), 13	30 (A->G), 31	15, 18	12, 12	9, 10	12, 12	16 (G->A), 21 (2A->2G)		
73	UNTHSC0034-M0378C1	11 (A->T), 13	30.2, 31	15, 16	12, 13 (G->T)	10, 10	12, 13 (A->G)	14 (A->G + 2T->2C), 14 (A->G + 2T->2C)	vWA	21 (2A-.2G)-> 20 (2A->2G)
74	UNTHSC0034-M0379A1	12, 13 (A->T)	31.2, 32.2	15 (2G->2A), 15 (G->A)	12, 12 (G->T)	11, 12	13, 13 (A->G)	16 (G->A), 17		
74	UNTHSC0034-M0379B1	8, 13	29 (G->A), 30	17 (G->A), 18	11, 13	10, 11	11, 12	15 (G->A), 19		
74	UNTHSC0034-M0379C1	12, 12	no data	15 (G->A), 18	12 (G->T), 13	11, 11	11, 13	15 (G->A), 16 (G->A)	D13S317	13->12
75	UNTHSC0034-M0380A1	11 (A->T), 13	32.2, 32.2	15, 18	9 (G->T), 12 (G->T)	10, 11	10, 12 (A->G)	18, 18		
75	UNTHSC0034-M0380B1	12, 13	28, 35 (T->G)	16 (G->A), 16 (G->A)	12, 13	10, 12 (T->A)	12 (A->G), 15 (A->G)	16, 18		
75	UNTHSC0034-M0380C1	12, 12	28, 31	15, 16 (G->A)	9 (G->T), 13	10, 12 (T->A)	10, 15 (A->G)	18, 18	D13S317	13->12
76	UNTHSC0034-M0381A1	12, 13	28, 28	16, 16 (2G->2A)	10, 12	6, 10	13 (A->G), 14 (A->G)	15, 16		
76	UNTHSC0034-M0381B1	11, 12 (A->T)	28, 30 (A->G)	15 (G->A), 18	10, 12	11, 13	12, 13 (A->G)	17, 17		
76	UNTHSC0034-M0381C1	13, 13 (A->T)	28, 28	16, 18	12, 12	10, 13	13 (A->G), 14 (A->G)	16, 17	D13S317	12 (A->T)-> 13 (A->T)
77	UNTHSC0034-M0382A1	11 (A->T), 12	28, 30 (A->G)	15 (G->A), 17 (2G->2A)	10, 11	8, 10	15 (2A->2G), 15 (2A->2G)	15, 18		
77	UNTHSC0034-M0382B1	11, 14	28, 35	16 (2G->2A), 16 (2G->2A)	12, 13	10, 11	14 (A->G), 14 (A->G)	17, 19		
77	UNTHSC0034-M0382C1	12, 14	28, 35	16 (2G->2A), 17 (2G->2A)	10, 13	10, 11	14 (A->G), 15 (2A->2G)	15, 18	vWA	17->18 or 19->18
78	UNTHSC0034-M0385A1	11 (A->T), 12	30 (A->G), 31.2	15 (2G->2A), 15 (2G->2A)	11, 12 (G->T)	8, 9	13 (A->G), 13 (A->G)	17, 17 (G->A)		
78	UNTHSC0034-M0385B2	11, 12 (A->T)	31.2, 31.2	15 (G->A), 17 (G->A)	12, 12 (G->T)	8, 11	11, 13 (A->G + C->G)	17, 18		
78	UNTHSC0034-M0385C1	11, 11 (A->T)	30 (A->G), 31.2	15 (2G->2A), 15 (G->A)	12 (G->T), 12 (G->T)	9, 11	12, 13 (A->G)	17, 18	D8S1179	11->12
79	UNTHSC0034-M0386A1	11, 11	30 (A->G), 31.2	9 (G->A), 15 (G->A)	13, 13	9, 9	11, 14 (A->G)	18, 18 (G->A)		
79	UNTHSC0034-M0386B1	9, 11 (A->T)	28, 30 (A->G)	15 (G->A), 17 (G->A)	10, 13	7, 10	14 (A->G), 16 (A->G)	16, 18 (A->G)		
79	UNTHSC0034-M0386C1	11, 11 (A->T)	30 (A->G), 31.2	15 (G->A), 17 (G->A)	13, 14	9, 10	14 (A->G), 16 (A->G)	18 (A->G), 18 (G->A)	D5S818	13->14
80	UNTHSC0034-M0388A1	11, 11 (A->T)	29, 33.2	15 (G->A), 16	12, 12 (G->T)	11, 12	10, 14 (A->G)	17, 18		
80	UNTHSC0034-M0388B1	11 (A->T), 12	28, 29	14 (G->A), 17 (G->A)	11, 11	8, 11	10, 12	14 (A->G + 2T->2C), 17		
80	UNTHSC0036-M0388C1	11, 11 (A->T)	28, 28	14 (G->A), 16	11, 12	11, 12	10, 14 (A->G)	14 (A->G + 2T->2C), 18	D21S11	29->28
81	UNTHSC0034-M0389A1	11, 11 (A->T)	29, 32.2	15 (G->A), 16	11, 12	10, 12	13 (A->G), 14	17, 17		
81	UNTHSC0034-M0389B1	11 (A->T), 13	29, 30.2	14 (2G->2A), 15 (2G->2A)	11, 11	10, 10	13 (A->G), 14 (A->G)	16, 18 (G->A)		
81	UNTHSC0034-M0389C2	11, 11 (A->T)	29, 30.2	15 (2G->2A), 16	11, 12	10, 12	13 (A->G), 14 (A->G)	17, 19 (G->A)	vWA	18 (G->A)-> 19 (G->A)
81	UNTHSC0036-M0389C1	11, 11 (A->T)	27, 32.2	16, 16 (G->A)	11, 12	10, 13	14, 16 (A->G)	17, 19 (A->G)		
82	UNTHSC0035-M0409A1	9, 10 (A->T)	29 (G->A), 31.2	15 (G->A), 15 (G->A)	11, 11 (G->T)	8, 11 (T->A)	14, 14 (A->G)	15 (A->G + 2T->2C), 18		
82	UNTHSC0035-M0409B1	9, 12	30, 32.2	17 (G->A), 18	11, 13	8, 12 (T->A)	13 (A->G), 14	16, 17		
82	UNTHSC0035-M0409C1	9, 10 (A->T)	29 (G->A), 32.2	15 (G->A), 17 (G->A)	11, 13	8, 11 (T->A)	13 (A->G), 14	15 (A->G + 2T->2C), 17		
82	UNTHSC0035-M0409C2	10 (A->T), 12	31.2, 32.2	15 (G->A), 18	11, 13	8, 8	13 (A->G), 14 (A->G)	18, 18	vWA	17->18
82	UNTHSC0035-M0409C3	9, 10 (A->T)	31.2, 32.2	15 (G->A), 17 (G->A)	11, 11	8, 11 (T->A)	14, 14 (A->G)	16, 18		
82	UNTHSC0035-M0409C4	10 (A->T), 12	30, 31.2	15 (G->A), 18	11, 11	8, 11 (T->A)	14, 14 (A->G)	15 (A->G + 2T->2C), 16		
83	UNTHSC0035-M0425A1	8, 12 (A->T)	30, 31	15 (G->A), 15 (G->A)	9 (G->T), 12 (G->T)	11, 11	13 (A->G), 14 (A->G)	16, 17		
83	UNTHSC0035-M0425B1	11 (A->T), 12	30 (A->G), 31.2 (G->A)	16, 17 (G->A)	7, 11	11, 12	12, 14 (A->G)	17, 18		
83	UNTHSC0035-M0425C1	12 (A->T), 12 (A->T)	30, 30 (A->G)	15 (G->A), 15 (G->A)	11, 12 (G->T)	11, 11	13 (A->G), 14 (A->G)	16, 16		
84	UNTHSC0035-M0392A1	12, 14	28, 28	15 (2G->2A), 16	11, 12	10, 12	14 (A->G), 15 (A->G)	16 (T->C), 18		
84	UNTHSC0035-M0392B1	11 (A->T), 12 (A->T)	28, 28	15 (G->A), 16 (G->A)	12 (G->T), 12 (G->T)	10, 10	14 (A->G), 15 (A->G)	15, 17		
84	UNTHSC0035-M0392C1	11 (A->T), 12	28, 33.2	15 (2G->2A), 16 (G->A)	10, 12	9, 12	15 (A->G), 15 (A->G)	16 (T->C), 17		
85	UNTHSC0035-M0394A1	11, 14	29 (G->A), 31	15 (G->A), 18	7, 11	10, 10	10, 15 (A->G)	15 (A->G + 2T->2C), 18		
85	UNTHSC0035-M0394B1	12, 12 (A->T)	31, 32.2	16 (2G->2A), 18 (G->A)	12, 13 (G->C)	10, 11	11 (A->G), 14 (A->G)	16, 17		
85	UNTHSC0035-M0394C1	13 (A->T), 14	29 (G->A), 31	16 (2G->2A), 18	7, 12	10, 10	11 (A->G), 15 (A->G)	16, 18	D13S317	12 (A->T)-> 13 (A->T)
86	UNTHSC0035-M0395A1	8, 11	32.2 (A->G), 33.2	15 (G->A), 17 (G->A)	11, 12	11, 12 (T->A)	12, 15 (A->G)	16, 17		
86	UNTHSC0035-M0395B1	8, 11	31, 32.2 (A->G)	15 (G->A), 17 (G->A)	11, 12	11, 12 (T->A)	12, 14 (A->G)	17, 18		
86	UNTHSC0035-M0395C1	11, 11	32.2 (A->G), 33.2	15 (G->A), 17 (G->A)	11, 11	11, 12 (T->A)	13, 15 (A->G)	17, 18	D8S1179	12->13
87	UNTHSC0035-M0396A1	9, 10	30 (A->G), 30 (A->G)	14 (G->A), 16 (G->A)	11, 11	11, 12	13, 13 (A->G)	16, 18		
87	UNTHSC0035-M0396B1	9, 9	30, 32.2	15 (G->A), 18	11, 12	11, 11 (T->A)	13, 13 (A->G)	15 (G->A), 17		
87	UNTHSC0035-M0396C2	9, 9	30 (A->G), 32.2	16 (G->A), 18	11, 11	11, 11 (T->A)	13, 13 (A->G)	16 (G->A), 18	vWA	15 (G->A)-> 16 (G->A)
88	UNTHSC0035-M0397A1	9, 10 (A->T)	28, 30 (A->G)	15 (G->A), 16 (G->A)	9 (G->T), 11	8, 12	10, 13 (A->G)	16, 18		
88	UNTHSC0035-M0397B1	11 (A->T), 13	29, 31.2	15 (G->A), 16 (G->A)	10, 11	11, 11 (T->A)	13, 15 (A->G)	17, 17		
88	UNTHSC0035-M0397C1	10 (A->T), 13	27 (A->G), 31.2	15 (G->A), 15 (G->A)	9 (G->T), 10	11, 12	10, 15 (A->G)	16, 17	D21S11	28->27
89	UNTHSC0035-M0398A1	11 (A->T), 12	31 (G->A), 32.2	14 (2G->2A), 17	13 (G->C), 13 (G->C)	9, 12	12 (A->G), 13 (A->G)	15, 15		
89	UNTHSC0035-M0398B1	11, 12	28, 31.2	14 (G->A), 15 (G->A)	12, 12 (G->T)	9, 11	14 (A->G), 14 (A->G)	15, 17		
89	UNTHSC0035-M0398C1	11 (A->T), 13	31 (G->A), 31.2	15 (G->A), 17	12, 13 (G->T)	9, 12	13 (A->G), 14 (A->G)	15, 15	D13S317	12->13
90	UNTHSC0035-M0400A1	8, 11 (A->T)	30, 31	15 (G->A), 18	11, 12	8, 10	13, 15 (A->G)	14 (A->G + 2T->2C), 17		
90	UNTHSC0035-M0400B1	12, 12 (A->T)	30 (A->G), 32	15 (2G->2A), 18	8 (G->T), 10	11, 13	16 (A->G), 16 (A->G)	18, 20 (A->G)		
90	UNTHSC0035-M0400C1	8, 12	31, 32	15 (G->A), 18	10, 12	8, 11	15 (A->G), 17 (A->G)	17, 18	D8S1179	16 (A->G)-> 17 (A->G)
91	UNTHSC0035-M0401A1	12, 12 (A->T)	27, 30 (A->G)	15 (2G->2A), 16	10, 11 (G->T)	8, 10	11, 13 (A->G)	19, 19		
91	UNTHSC0035-M0401B1	13, 13	31.2, 32.2	14 (2G->2A), 15 (G->A)	12, 12	11, 13 (T->A)	15 (2A->2G + A->G), 16 (A->G)	15, 16 (G->A)		
91	UNTHSC0035-M0401C1	12, 12 (A->T)	27, 32.2	14 (2G->2A), 15 (2G->2A)	11 (G->T), 12	10, 13 (T->A)	11, 15 (2A->2G + A->G)	15, 19	D13S317	13->12
92	UNTHSC0035-M0402A1	10 (A->T), 12	29, 32.2	15 (G->A), 16	11, 12	12, 13	15 (A->G), 16 (A->G)	16, 16 (G->A)		
92	UNTHSC0035-M0402B1	8, 10 (A->T)	31.2, 32 (A->G)	15 (G->A), 17	11, 11	9, 12 (T->A)	11, 13 (A->G)	16, 17		
92	UNTHSC0035-M0402C1	10 (A->T), 12	31.2, 32.2	15 (G->A), 17	11, 11	9, 13	13 (A->G), 15 (A->G)	16, 16		
93	UNTHSC0035-M0403A1	12, 13 (A->T)	29, 33.2	15 (G->A), 16 (G->A)	11, 13	10, 11	12, 15 (2A->2G)	17, 18		
93	UNTHSC0035-M0403B1	12, 12	30 (A->G), 35.2	16, 16 (G->A)	7, 11	10, 11	10, 15 (A->G)	17, 19		
93	UNTHSC0035-M0403C1	11, 13 (A->T)	30 (A->G), 33.2	15 (G->A), 16 (G->A)	11, 13	10, 10	10, 12	17, 17	D13S317	12->11
94	UNTHSC0035-M0404A1	12, 12 (A->T)	32, 32.2	15 (G->A), 16 (2G->2A)	11, 12	10, 12	13 (A->G), 13 (A->G)	17 (G->A), 19		
94	UNTHSC0035-M0404B1	8, 12	29 (A->G), 31	15 (2G->2A), 18	9 (G->T), 11	9, 9	10, 13 (A->G)	16, 18		
94	UNTHSC0035-M0404C1	12, 12 (A->T)	31, 32	16 (2G->2A), 18	9 (G->T), 12	9, 12	11, 13 (A->G)	16, 17 (G->A)	D8S1179	10->11
95	UNTHSC0035-M0405A1	10 (A->T), 13	29, 31.2	16 (G->A), 18 (G->C)	11, 11	12, 14 (T->A)	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 19		
95	UNTHSC0035-M0405B1	9, 11 (A->T)	30, 32.2	16 (G->A), 17	9 (G->T), 11	10 (T->A), 12	13 (A->G), 14	15 (G->A), 17		
95	UNTHSC0035-M0405C1	9, 13	29, 30	16 (G->A), 18 (G->C)	11, 11	12, 14 (T->A)	13 (A->G), 14	14 (A->G + 2T->2C), 16 (G->A)	vWA	15 (G->A)-> 16 (G->A)
95	UNTHSC0035-M0405C2	8, 13	29, 31.2	15 (G->A), 16 (G->A)	11, 12	11, 14 (T->A)	12, 13 (A->G)	16, 19		
95	UNTHSC0035-M0405C3	9, 10 (A->T)	29, 30	17, 18 (G->C)	11, 11	10 (T->A), 12	13 (A->G), 13 (A->G)	14 (A->G + 2T->2C), 15 (G->A)		
96	UNTHSC0035-M0406A1	11, 11 (A->T)	30 (A->G), 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	9, 11	13 (A->G), 14 (A->G)	16, 18		
96	UNTHSC0035-M0406B1	9, 9	28, 29	15 (G->A), 18	11, 11	8, 12 (T->A)	11, 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C1	9, 11	28, 30 (A->G)	15 (G->A), 18	11, 11	9, 12 (T->A)	11, 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C2	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	8, 9	11, 13 (A->G)	16 (G->A), 18		
96	UNTHSC0035-M0406C3	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	7, 11	11, 12 (T->A)	13 (A->G), 14 (A->G)	16, 16 (G->A)		
96	UNTHSC0035-M0406C4	9, 11	29, 30 (A->G)	15 (G->A), 15 (G->A)	11, 11	8, 9	14 (A->G), 14 (A->G)	16, 17 (G->A)	D8S1179	16 (A->G)-> 17 (A->G)
97	UNTHSC0036-82001A1	11 (A->T), 12	28, 30	14 (G->A), 17 (2G->2A)	11, 12 (G->T)	8, 12	12, 12 (A->G)	16, 18		
97	UNTHSC0036-82001B1	8, 8	28, 34 (2A->2G)	15 (G->A), 16 (G->A)	11, 12	8, 10	11, 13	15, 17 (G->A)		
97	UNTHSC0036-82001C1	8, 12	28, 34 (2A->2G)	14 (G->A), 15 (G->A)	11, 11	8, 10	11, 12	15, 18		
97	UNTHSC0036-82001C2	8, 12	28, 30	14 (G->A), 16 (G->A)	11, 12 (G->T)	8, 10	11, 12 (A->G)	15, 16		
97	UNTHSC0036-82001C3	8, 12	28, 30	14 (G->A), 16 (G->A)	12, 12 (G->T)	8, 12	12, 13	15, 18		
97	UNTHSC0036-82001C4	8, 12	28, 34 (2A->2G)	16 (G->A), 17 (2G->2A)	11, 11	8, 8	12 (A->G), 13	15, 18		

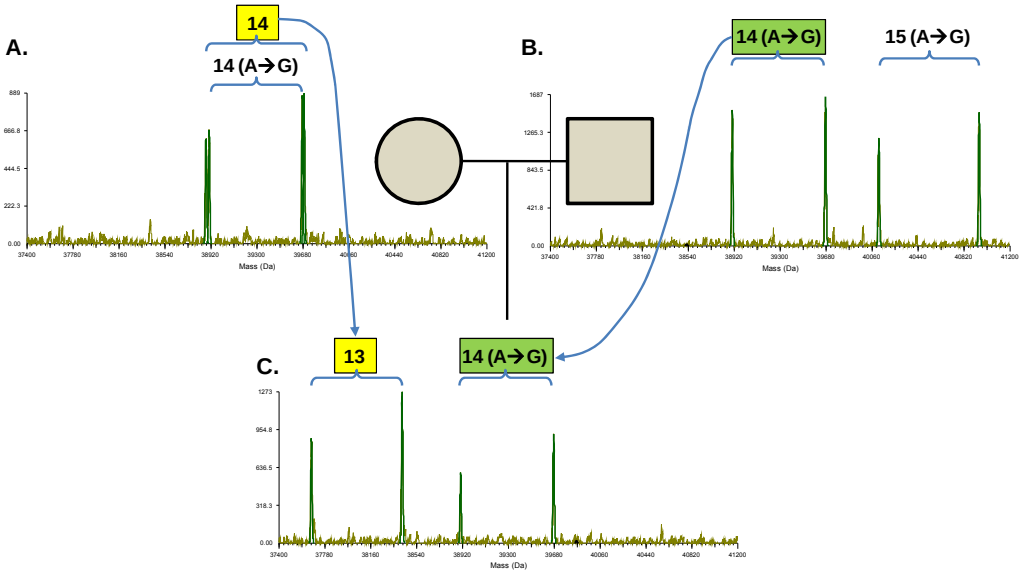


Figure 48. D8S1179 genotypes for a mother-father-child trio. The mother (A.) has genotype 14, 14 (A→G). The father (B) has genotype 14 (A→G), 15 (A→G). The child (C) has genotype 13, 14 (A→G). The simplest explanation for this observation is that the father contributed the non-mutated 14 (A→G) allele and the mother in fact contributed an allele 14 that mutated to a 13 in the child.

Specific Aim 5: Continued development of transferable analysis software with an intuitive user interface

5.1 Complete the STR assay data processing automation

Over the course of this project, the processing of raw mass spectra to produce deconvolved spectral traces and numerical mass and intensity values that are utilized during the data analysis of STR and Y-STR assay outputs has progressed from a manually-triggered interface running within Matlab to a fully-automated, completely native code-based processing application written in C# (no Matlab interface or runtime environment required) that requires no user input and is seamlessly integrated in the process of running on a plate on the Ibis instrument. Recently, the processing application has been migrated to a Windows service module that runs as a background process on the data processing server and is invisible to the user. After thermocycling an assay plate that has been registered into the IbisTrack database, the assay plate is placed upon the Ibis T5000 or PLEX-ID instrument and the instrument is started. At the point that all data has been collected for the plate, the automation controller automatically converts the data into a familiar folder-based data output, copies all raw spectral data to a configured output directory, triggers the processing of raw mass spectra into deconvolved mass spectra, generates and output list of masses and signal intensities, and imports the output back into the database linked to the barcode of the assay plate to await analysis and visual QC. This process operates identically for all forensics applications. The progression of the raw data processing component of this system was as follows:

1. Development of a novel TOF-based mass spectral deconvolution algorithm for ESI-analyzed DNA molecules of moderate size (~12 kDa and larger) called MassCollapse.
2. Implementation and testing of MassCollapse in Matlab in combination with the existing mass spectral calibration routines used in the Ibis biosensor data processing algorithms.
3. Development of a manual interface to allow assay plate-based data processing of STR data and an interface within the forensics data analysis module allowing for the manual import of processed spectral data into the Oracle database (Figure 49).
4. Refactoring of prototype code used in the process triggered manually as in Figure 49, A and compilation into a Matlab runtime-utilizable library triggered automatically by the Ibis T5000 automation controller.

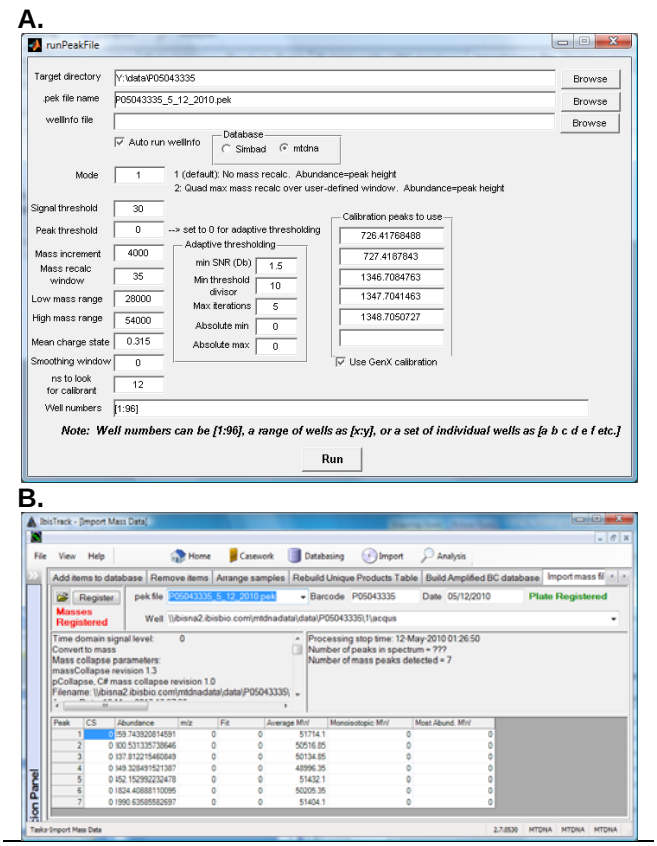


Figure 49. Manual data processing implementation. A. Matlab interface for processing plate-based mass spectral data. B. Interface within forensics data analysis module allowing manual import of spectral processing data output into the Oracle database.

5. Exposure of a method within the forensics analysis module (.dll file) allowing the Ibis T5000 controller to trigger automated import of processed spectral data into the Oracle database after data processing.
6. Development of a Graphical User Interface-based data processing monitor (Figure 50) that allows a user to check the status of data processing or trigger/retrigger plate processing in the event of a network interruption or workflow anomaly.
7. Incremental conversion of Matlab-based processing code-based to completely native Microsoft C# code base. This includes an organized C# port of a large proportion of the basic Matlab mathematical and signal processing libraries.

Conversion to C# is complete and spectral processing runs completely in C# code now (no Matlab runtime installation required).

8. Repackaging automated data processing application into a native Windows service that can run in the background. This is now running in production at Ibis Biosciences to support our service laboratory (renamed as Athogen and relocated to Irvine, CA).

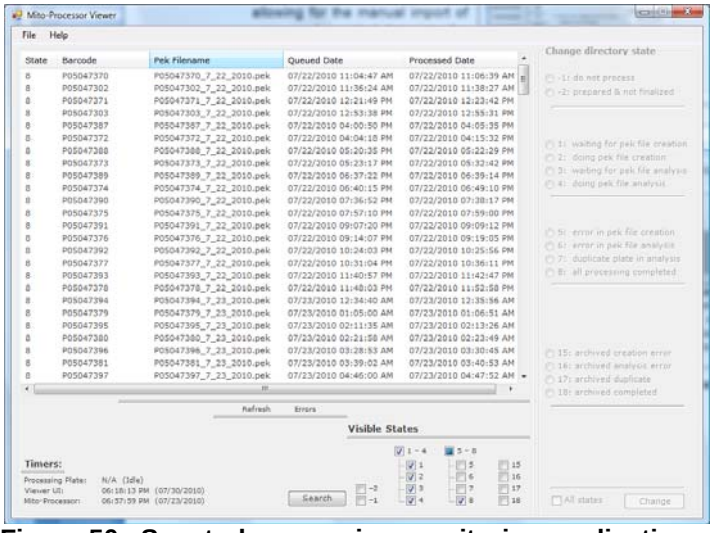


Figure 50. Spectral processing monitoring application.

5.2. Refine the STR analysis interface

Over the course of this project, the forensics data analysis interface and database has been generalized to allow for the analysis of any base composition or allele-based forensic assay running on the Ibis platform including mtDNA profiling, STRs, Y-STRs or autosomal SNP markers. The processing and analysis mode is easily extendable to other forensic-type analysis such as microbial SNP or VNTR analysis, as the analysis mode and profiling methodology is essentially the same as that done with human DNA. Functionality to store and retrieve STR and Y-STR profiles directly from the analysis interface has been implemented, as well as an interface to search STR or Y-STR profiles using a stored profile as a query (Figure 51).

In addition to the automated analysis of STR and Y-STR profiles in a framework with many of the features of an “Expert system”, several enhancements have been made to the data viewer. For example, an automated deconvolved data trace noise baseline subtraction is implemented, the option to “auto-zoom” to the relevant x-axis coordinated for assigned allele products within a PCR reaction, addition of color-coded allele assignment labels in the deconvolved spectral data view, and the ability to browse allele hypothesis directly within the deconvolved data viewer to aid analysis of problematic samples. The STR analysis interface for both the Ibis T5000 and PLEX-ID will undergo continued refinement and is currently undergoing a complete rewrite that will be implemented in the future. However, Abbott Molecular is currently undergoing commercialization of the forensics PLEX-ID system and the forensics analysis software is on the verge of commercialization.

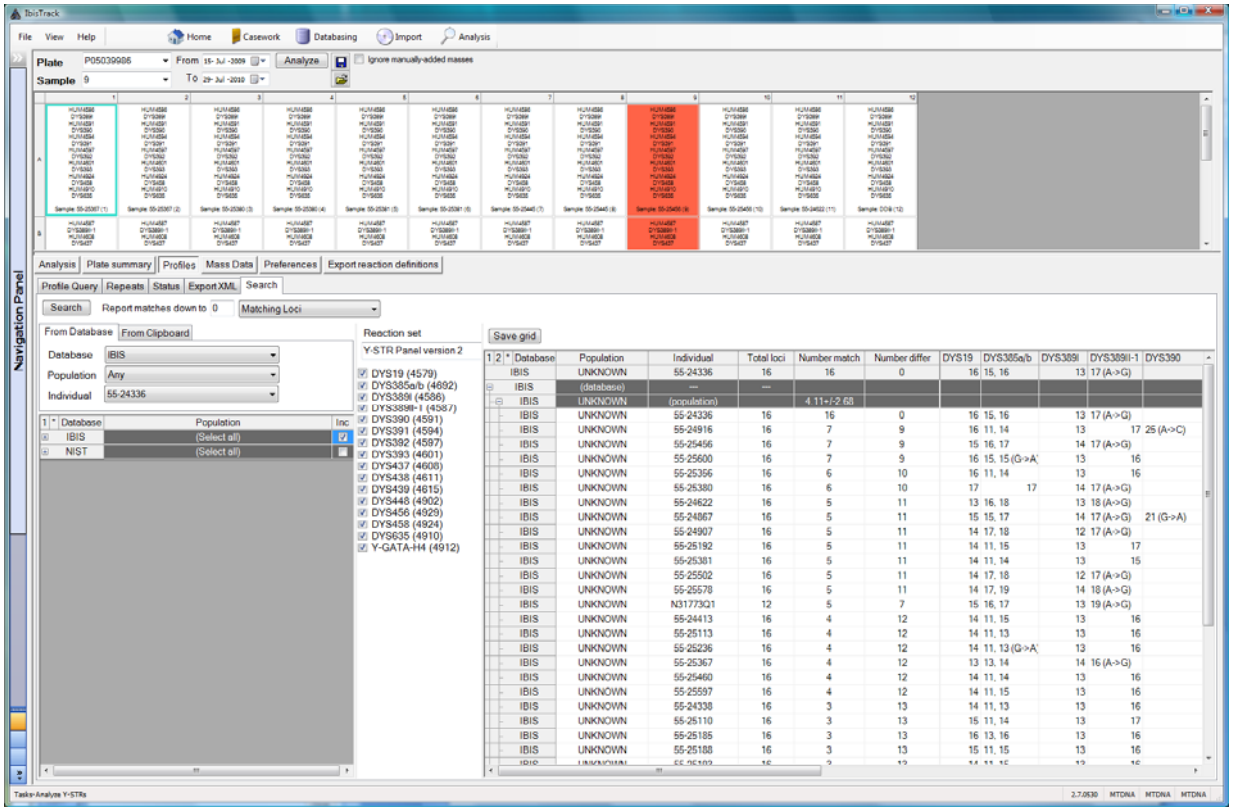


Figure 51. Search interface showing the search of a stored Y-STR profile.

ESI-TOF-MS platform-specific considerations

Operation of the PLEX-ID™ instrument

User level expectation

The Ibis PLEX-ID™ analyzer is a novel instrumentation platform that utilizes electrospray ionization (ESI) analyte preparation integrated to a time of flight (TOF) mass analyzer. Although TOF analysis of forensic DNA markers is not a new concept, and several studies have employed MALDI-TOF-MS[32-37] or ESI-TOF-MS[71-74] for the analysis of forensic DNA markers. The novelty of the Ibis PLEX-ID™ analyzer lies in the end-to-end automation and simplicity of use for a high-precision analytical platform. Although it admittedly takes a trained technician to service a compromised instrument, the day-to-day operation of a PLEX-ID™ requires only a technician with minimal training on the instrument and no formal background in mass spectrometry or analytical chemistry. Running parameters are fully locked down and integrated in the automated system. To operate a PLEX-ID™, the technician simply needs to know how to check and fill the reagent bottles with numbered liquid reagents, load post-amplified and still-sealed 96-well PCR plates into plate stacker, and press the "Run" button on the touch screen. All subsequent steps, including DNA desalination and desolvation, data collection, and data processing, are fully automated. The instrument can run 15 96-well plates in an uninterrupted run. Data analysis for STRs and Y-STRs is nearly completely automated, but involves a manual step of launching the analysis software and choosing the barcode of the plate(s) to be analyzed. An Intuitive data interrogation interface with

integrated and dynamic graphical tools allows manual evaluation of data to forensic scientists who do not need to be experts in mass spectrometry.

Analysis time

The PLEX-ID™ system analyzes one PCR reaction approximately every 30 seconds. If the system is idle, at the time one or more plates is set on the machine and the system is started, there is a brief initialization and priming period, then PCR reactions are loaded sequentially into a revolving carousel of cuvettes for clean-up/preparation for mass spectrometry analysis. It takes about 15 minutes for the first cleaned reaction to get to the point of injection into the mass spectrometer, and then reactions are analyzed continuously at a rate of 30 seconds per reaction. The first 96-well plate in a run take just over an hour, and subsequent 96-well plates will take approximately 50 minutes each. Data processing occurs automatically subsequent to mass spectrometry data acquisition, takes approximately 15-20 minutes per plate, and runs in parallel with data acquisition for the next plate. The current STR assay requires about three hours of thermocycling time. After transferring DNA to a PCR plate, therefore, the minimum time from placing a plate into the thermocycler and having data ready for analysis is about 4.5 hours. Downstream analysis time for processed data will vary depending on data quality and sample complexity. For single-source samples yielding high-quality data, analysis time can be about 5-10 minutes for a plate of 12 samples. For a databasing application employing five thermocyclers in parallel, five PCR plates could be thermocycled in parallel, loaded on the PLEX-ID™, then followed by five more plates. Three cycles of this would take 9-10 hours, including manipulation of the plates. The 15 plates would require a total continuous time of approximately 12.75 hours. With 1-3 hours of analysis time for the 15 plates, this would be equivalent to 180 samples in about 15 hours. This does not include DNA sample preparation, which is an independent step outside the scope of this report.

Limitations of the ESI-TOF-MS method

While the ESI-TOF-MS system described herein offers the advantages of convenience (no manual manipulations after adding template), simplicity (no allelic ladders required), wide template input operating range (upper limit of DNA template that can be added is enormous) and enhanced information content (polymorphisms are revealed in forensic markers), it is not without limitations that may be important depending on the sample(s) being analyzed. The ESI method produces an entire distribution of detected signals for each analyte due to the fact that a distribution of each molecule is prepared containing different numbers of negative charges. Because each specific molecule in a reaction produces multiple signals, multiplexed products quickly produce a very congested spectrum, making it unfeasible to multiplex more than a small number of PCR primer pairs into a single reaction. Because of this limitation, a sample must be divided into several wells (eight in the current assay layouts) to cover all of the forensic markers in the assay. This ultimately limits the lower level of sensitivity such

that eight times the lower limit of PCR sensitivity is required for a full sample analysis, or >=1 ng of template.

Related to this limitation is a limitation imposed by the attempt to balance multiplex-induced spectral congestion with assay sensitivity and cost, which results in a higher overall noise baseline in spectra than could be achieved with monoplexed reactions. This limits the reliable dynamic range observed in mixed-template reactions. This is an areas where active efforts are being undertaken to improve performance, but presently, the dynamic range between major and minor products in a mixed sample is inferior to that achievable in current CGE systems. The other primary limitation of the ESI-MS methodology is the upper bound of product size that can be reliably analyzed. This is due to the spectral complexity resulting from higher numbers of charge states that are produced with large DNA fragments as well as a break-down of the mathematics involved in inferring an unambiguous product base composition from the forward and reverse strand masses of a PCR product (too many A, G, C, T combination possibilities occur for very large masses). For STR PCR products, which have a very constrained set of composition possibilities, it is not necessary in general to calculate a base composition exclusively from product masses without reference to a constrined range of possibilities. We have successfully analyzed STR PCR products pproaching 300 bp. In general, the ESI-MS system operates optimally with products <= 150 bp.

Although the ESI-MS method reveals the presence of polymorphisms within an STR locus, it is not able to locate where in the sequence a polymorphism occurs. Through sequencing a small number of selected alleles, we (and others) have demonstrated that there are sometimes SNPs within the flanking regions surrounding the repeat structure itself, which is the case for D7S820, which has an A←→T variation just downstream of the repeat that is resolved with the ESI-MS method. Often the polymorphism(s) is/are within the repeats themselves, turning a simple repeat into a complex repeat. Just as two allele 13's determined by CGE might in fact be different (13 vs. 13 (A->G), for example), it is also possible that, when determined with ESI-MS, two allele 13 (A->G) alleles may in fact be different, with the A->G SNP occurring in a different repeat unit in the two alleles. This would not be resolved in the ESI-MS system and both would be genotyped as the same 13 (A->G) allele. Likewise, the presence of two cancelling mutations will not be seen at all in the ESI system. For example, an allele 13 (A->G + G->A) (which is conceivably possible, albeit expected to be quite rare), would simply be detected as a nominal allele 13 with the ESI-MS system.

Instrument cost

The cost of a PLEX-ID™ system will vary depending on geopolitical region. For information regarding system pricing, refer to the PLEX-ID information site at http://plex-id.com/static/gateway.html to navigate to the proper geographic region and then follow the "Contact Us" link.

References

(1) Kumar, S.; Subramanian, S. *Proc Natl Acad Sci U S A* **2002**, *99*, 803-808.
(2) Kondrashov, A. S. *Hum Mutat* **2003**, *21*, 12-27.
(3) Weber, J. L.; Wong, C. *Hum Mol Genet* **1993**, *2*, 1123-1128.
(4) Zhivotovsky, L. A.; Underhill, P. A.; Cinnioglu, C.; Kayser, M.*, et al. Am J Hum Genet* **2004**, *74*, 50-61.
(5) Kruglyak, S.; Durrett, R. T.; Schug, M. D.; Aquadro, C. F. *Proc Natl Acad Sci U S A* **1998**, *95*, 10774-10778.
(6) Borstnik, B.; Pumpernik, D. *Genome Res* **2002**, *12*, 909-915.
(7) Pumpernik, D.; Oblak, B.; Borstnik, B. *Mol Genet Genomics* **2007**.
(8) Kayser, M.; Roewer, L.; Hedman, M.; Henke, L.*, et al. Am J Hum Genet* **2000**, *66*, 1580-1588.
(9) Butler, J. M. *Forensic Sci Rev* **2003**, *15*, 91-111.
(10) Delfin, F. C.; Madrid, B. J.; Tan, M. P.; De Ungria, M. C. *Int J Legal Med* **2005**, *119*, 158-163.
(11) Sibille, I.; Duverneuil, C.; Lorin de la Grandmaison, G.; Guerrouache, K.*, et al. Forensic Sci Int* **2002**, *125*, 212-216.
(12) Prinz, M.; Ishii, A.; Coleman, A.; Baum, H. J.*, et al. Forensic Sci Int* **2001**, *120*, 177-188.
(13) Prinz, M.; Boll, K.; Baum, H.; Shaler, B. *Forensic Sci Int* **1997**, *85*, 209-218.
(14) Honda, K.; Roewer, L.; de Knijff, P. *J Forensic Sci* **1999**, *44*, 868-872.
(15) Dekairelle, A. F.; Hoste, B. *Forensic Sci Int* **2001**, *118*, 122-125.
(16) Corach, D.; Filgueira Risso, L.; Marino, M.; Penacino, G.*, et al. Forensic Sci Int* **2001**, *118*, 131-135.
(17) Betz, A.; Bassler, G.; Dietl, G.; Steil, X.*, et al. Forensic Sci Int* **2001**, *118*, 126-130.
(18) Jobling, M. A. *Trends Genet* **2001**, *17*, 353-357.
(19) Sykes, B.; Irven, C. *Am J Hum Genet* **2000**, *66*, 1417-1419.
(20) Rolf, B.; Keil, W.; Brinkmann, B.; Roewer, L.*, et al. Int J Legal Med* **2001**, *115*, 12-15.
(21) Jobling, M. A.; Pandya, A.; Tyler-Smith, C. *Int J Legal Med* **1997**, *110*, 118-124.
(22) Dettlaff-Kakol, A.; Pawlowski, R. *Int J Legal Med* **2002**, *116*, 289-291.
(23) Butler, J. M. *Forensic DNA Typing*, 2nd ed.; Elsevier Academic Press: Burlington, 2005.
(24) Graves, J. A.; Wakefield, M. J.; Toder, R. *Hum Mol Genet* **1998**, *7*, 1991-1996.
(25) Schneider, P. M.; Bender, K.; Mayr, W. R.; Parson, W.*, et al. Forensic Sci Int* **2004**, *139*, 123-134.
(26) Butler, J. M.; Shen, Y.; McCord, B. R. *J Forensic Sci* **2003**, *48*, 1054-1064.
(27) Gill, P.; Fereday, L.; Morling, N.; Schneider, P. M. *Forensic Sci Int* **2006**, *156*, 242-244.
(28) Coble, M. D.; Butler, J. M. *J Forensic Sci* **2005**, *50*, 43-53.
(29) Holland, M. M.; Cave, C. A.; Holland, C. A.; Bille, T. W. *Croat Med J* **2003**, *44*, 264-272.
(30) Hill, C. R.; Kline, M. C.; Mulero, J. J.; Lagace, R. E.*, et al. J Forensic Sci* **2007**, *52*, 870-873.
(31) Gusmao, L.; Alves, C.; Costa, S.; Amorim, A.*, et al. Int J Legal Med* **2002**, *116*, 322-326.

(32) Sanchez, J. J.; Borsting, C.; Hallenberg, C.; Buchard, A.*, et al. Forensic Sci Int* **2003**, *137*, 74-84.
(33) Vallone, P. M.; Decker, A. E.; Butler, J. M. *Forensic Sci Int* **2005**, *149*, 279-286.
(34) Sobrino, B.; Brion, M.; Carracedo, A. *Forensic Sci Int* **2005**, *154*, 181-194.
(35) Armstrong, B.; Stewart, M.; Mazumder, A. *Cytometry* **2000**, *40*, 102-108.
(36) Harrisona, C.; Musgrave-Browna, E.; Benderb, K.; Carracedoc, A.*, et al. Int. Congress Ser.* **2006**, *1288*, 52-54.
(37) Paracchini, S.; Arredi, B.; Chalk, R.; Tyler-Smith, C. *Nucleic Acids Res* **2002**, *30*, e27.
(38) Butler, J.; Becker, C. H. *Science and Technology Research Report to NIJ* **2001**, *NCJ 188292*, October.
(39) Monforte, J. A.; Becker, C. H. *Nat Med* **1997**, *3*, 360-362.
(40) Taranenko, N. I.; Golovlev, V. V.; Allman, S. L.; Taranenko, N. V.*, et al. Rapid Commun Mass Spectrom* **1998**, *12*, 413-418.
(41) Butler, J. M.; Li, J.; Shaler, T. A.; Monforte, J. A.*, et al. Int J Legal Med* **1999**, *112*, 45-49.
(42) Ross, P. L.; Belgrader, P. *Anal Chem* **1997**, *69*, 3966-3972.
(43) Braun, A.; Little, D. P.; Reuter, D.; Muller-Mysok, B.*, et al. Genomics* **1997**, *46*, 18-23.
(44) Braun, A.; Little, D. P.; Koster, H. *Clin Chem* **1997**, *43*, 1151-1158.
(45) Hofstadler, S. A.; Sampath, R.; Blyn, L. B.; Eshoo, M. W.*, et al. Inter. J. Mass Spectrom.* **2005**, *242*, 23-41.
(46) Hofstadler, S.; Drader, J.; Schink, A. *Anal Chem* **2006**, *78*, 372-378.
(47) Ruitberg, C. M.; Reeder, D. J.; Butler, J. M. *Nucleic Acids Res* **2001**, *29*, 320-322.
(48) Hofstadler, S. A.; Hall, T. A. *NIJ final report* **2008**, *Contract Number #2006-DN-BX-K011*.
(49) Clark, J. M. *Nucleic Acids Res* **1988**, *16*, 9677-9686.
(50) Hu, G. *DNA Cell Biol* **1993**, *12*, 763-770.
(51) Magnuson, V. L.; Ally, D. S.; Nylund, S. J.; Karanjawala, Z. E.*, et al. Biotechniques* **1996**, *21*, 700-709.
(52) Brownstein, M. J.; Carpten, J. D.; Smith, J. R. *Biotechniques* **1996**, *20*, 1004-1006, 1008-1010.
(53) Butler, J. M.; Schoske, R.; Vallone, P. M.; Redman, J. W.*, et al. J Forensic Sci* **2003**, *48*, 908-911.
(54) Henegariu, O.; Heerema, N. A.; Dlouhy, S. R.; Vance, G. H.*, et al. Biotechniques* **1997**, *23*, 504-511.
(55) Kainz, P. *Biochim Biophys Acta* **2000**, *1494*, 23-27.
(56) Blanchard, M. M.; Taillon-Miller, P.; Nowotny, P.; Nowotny, V. *PCR Methods Appl* **1993**, *2*, 234-240.
(57) Gelfand, D. H.; White, T. J. In *PCR Protocols. A Guide to Methods and Applications, First ed.*; Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J., Eds.; Academic Press, Inc.: San Diego, 1990, pp 129-141.
(58) Baskaran, N.; Kandpal, R. P.; Bhargava, A. K.; Glynn, M. W.*, et al. Genome Res* **1996**, *6*, 633-638.
(59) Frackman, S.; Kobs, G.; Simpson, D.; Storts, D. *Promega Notes* **1998**, *27*.
(60) Musso, M.; Bocciardi, R.; Parodi, S.; Ravazzolo, R.*, et al. J Mol Diagn* **2006**, *8*, 544-550.
(61) Sarkar, G.; Kapelner, S.; Sommer, S. S. *Nucleic Acids Res* **1990**, *18*, 7465.

(62) Hall, T. A.; Sannes-Lowery, K. A.; McCurdy, L. D.; Fisher, C.*, et al. Anal Chem* **2009**.
(63) Ayub, Q.; Mohyuddin, A.; Qamar, R.; Mazhar, K.*, et al. Nucleic Acids Res* **2000**, *28*, e8.
(64) Wu, F. C.; Pu, C. E. *Forensic Sci Int* **2001**, *120*, 213-222.
(65) Furedi, S.; Woller, J.; Padar, Z.; Angyal, M. *Int J Legal Med* **1999**, *113*, 38-42.
(66) Schneider, P. M.; Meuser, S.; Waiyawuth, W.; Seo, Y.*, et al. Forensic Sci Int* **1998**, *97*, 61-70.
(67) Appendix *Int J Legal Med* **1997**, *110*, 141-149.
(68) Dupuy, B. M.; Gedde-Dahl, T.; Olaisen, B. *Forensic Sci Int* **2000**, *112*, 111-121.
(69) Mayntz-Press, K. A.; Ballantyne, J. *J Forensic Sci* **2007**, *52*, 1025-1034.
(70) Budowle, B.; Ge, J.; Aranda, X. G.; Planz, J. V.*, et al. J Forensic Sci* **2009**.
(71) Oberacher, H.; Parson, W.; Muhlmann, R.; Huber, C.G. *Anal Chem* **2001**, *73*, 5109-5115.
(72) Oberacher, H.; Niederstatter, H.; Parson, W. *Int J Legal Med* **2007**, *121*, 57-67.
(73) Oberacher, H.; Niederstatter, H.; Pitterl, F.; Parson, W. *Anal Chem* **2006**, *78*, 7816-7827.
(74) Oberacher, H.; Pitterl, F.; Huber, G.; Niederstatter, H.; Steinlechner, M.; Parson, W. *Hum Mutat* **2008**, *29*, 427-432.

Dissemination

Since the beginning of the phase I phase of this effort, we have collaborated with members of the DNA Unit II Laboratory division at the FBI, Quantico, VA, in a successful effort to transfer this technology into the hands of forensic scientists outside of our own laboratory. The FBI DNA Unit II (mitochondrial analysis division) purchased two Ibis T5000 instruments and multiple mtDNA tiling kits for use with the mitochondrial control region tiling assay and has recently inquired about the Ibis PLEX-ID. We previously held four hands-on training sessions with FBI staff members and have transferred detailed SOPs and full software user's manuals to FBI forensics scientists.

An Ibis T5000 instrument has been purchased and placed in the laboratory of John Planz at the University of North Texas Health Sciences Center (UNTHSC). Ibis PLEX-ID instruments have been purchased and placed at the Armed Forces DNA Identification Laboratory (AFDIL), three branches of the FBI regional crime laboratories (New Jersey, Minnesota and Arizona) and the Australian Federal Police (AFP). We have published the primary description of the Ibis mitochondrial profiling assay[62] (in collaboration with members of the FBI DNA Unit II,) developed in part during the phase I effort project #2006-DN-BX-K011. We are currently collaborating with John Planz at UNTHSC to publish the description of the STR assay and preliminary population screening of polymorphic allele frequencies. In cooperation with the National Forensic Science Technology Center (NFSTC), we have hosted two and contributed to one

technology transfer workshop. Portions of this work have also been presented at multiple conferences.

Seminars, workshops and conferences where portions of this work have been presented:

1. Analysis of DNA Forensic Markers Using High Throughput Mass Spectrometry. Thomas Hall, Sheri Manalili, Kristin Sannes-Lowery, Almira Henthorne, Jessica Paulsen, Amy Schink, Leslie McCurdy, Thuy Tran-Pennella, Lora Gioeni, Bruce Budowle and Steven A. Hofstadler, Poster presented by Thomas Hall at the 2008 NIJ Conference, July 21-23, 2008, Arlington, VA.
2. Mitochondrial and STR DNA Analysis by Mass Spectrometry Using the Ibis Biosciences, Inc. Platform. Technology Transition Workshop facilitated by the NFSTC. Held February 9–11, 2009, at the Residence Inn, 2000 Faraday Ave, Carlsbad, CA 92008. Workshop presentations available online at http://projects.nfstc.org/tech_transition/ibis_2009/index.htm.
3. Analysis of DNA Forensic Markers Using High-Performance Mass Spectrometry. Thomas Hall, Kristin Sannes-Lowery, Sheri Manalili, Maria Tobar, Jessica Paulsen, Amy Schink, David Duncan, John Planz and Steven A. Hofstadler. Poster presented by Thomas Hall at the 2009 NIJ Conference, June 15-17, 2009, Arlington, VA.
4. Analysis of DNA Forensic Markers Using High Throughput Mass Spectrometry. Steven A. Hofstadler, Thomas A. Hall, Kristin A. Sannes- Lowery, Sheri Manalili, Jessica E. Paulsen, Leslie D McCurdy, Lora Gioeni, Thuy Penella, Arthur J. Eisenberg, John V. Planz and Bruce Budowle. Poster presented by Thomas Hall at the 23rd World Congress International Society for Forensic Genetics (ISFG), September 14-18, 2009, Buenos Aires, Argentina.
5. Forensic SNP Analysis. Technology Transition Workshop facilitated by the NFSTC. Held November 2-4, 2009, at the University of North Texas Health Sciences Center (UNTHSC), Fort Worth, TX.
6. Ibis Biosciences DNA Forensics Assays. Presented by Thomas Hall to CALDOJ, Feb 3, 2010 in Oakland, CA.
7. STR and Mitochondrial DNA Analysis by Mass Spectrometry For Managers Using the Ibis™ Biosciences, Inc. Platform. Technology Transition Workshop facilitated by the NFSTC. Held April 30, 2010, at Ibis Biosciences, 2251 Faraday Ave, Carlsbad, CA 92008.
8. Analysis of DNA Forensic Markers Using High-Performance Mass Spectrometry. Thomas A. Hall, David D. Duncan, Maria A. Tobar, Kristin Sannes-Lowery, Sheri M. Manalili, Jessica E. Paulsen and Steven A. Hofstadler. Software demonstration presented by Thomas Hall at the 2010 NIJ Conference, June 14-16, 2010, Arlington, VA.

9. Forensic Markers Using High Throughput Mass Spectrometry. Presented by Steven Hostadler at the 20th International Symposium on the Forensics Sciences hosted by the Australia and New Zealand Forensic Science Society (ANZFSS), September 7, 2010, Sydney, Australia.

10. Electrospray Ionization Mass Spectrometry for mtDNA and STR Profiling. Oral Presentation. Presented by Thomas Hall September 20, 2010 at the 2nd Annual Current and Future Advances in Human Identification Conference in Hampton, VA.
11. Analysis of DNA Forensic Markers (and SNPs) Using High-Performance Mass Spectrometry. Oral presentation. Presented by Thomas Hall October 10, 2010, at The 21st International Symposium on Human Identification satellite workshop for SNP analysis, hosted by Promega in San Antonio, TX.
12. Analysis of DNA Forensic MarkersUsing High-Performance MassSpectrometry. . Oral presentation. Presented by Thomas Hall October 13, 2010, at The 21st International Symposium on Human Identification, hosted by Promega in San Antonio, TX.
13. Developmental Validation of an STR Genotyping Assay Providing Base Composition Analysis by PCR/Electrospray Ionization Mass Spectrometry. D.D. Duncan, J.V. Planz, C.V. Marzan, M.A. Tobar, S.A. Hofstadler, and T.A. Hall. Poster presented October 13, 2010, by Thomas Hall at the 21st International Symposium on Human Identification, hosted by Promega in San Antonio, TX.