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Enhanced Kinship Analysis and STR-based DNA Typing for Human Identification in Mass Fatality Incidents: The Swissair Flight 111 Disaster*

ABSTRACT: A bioinformatic tool was developed to assist with the victim identification initiative that followed the Swissair Flight 111 disaster. Making use of short tandem repeat (STR) DNA typing data generated with AmpF/STR® Profiler Plus™ (PP) and AmpF/STR® COfiler™(CO) kits, the software systematically compared each available STR genotype with every other genotype. The matching algorithm was based on the search for: (i) direct matches to genotypes derived from personal effects; and (ii) potential kinship associations between victims and next-of-kin, as measured by allele sharing at individual loci. The software greatly assisted parentage analysis by enabling kinship evaluation in situations where complete parentage trios were unavailable and, in some situations, with distantly related relatives. Exclusion of fortuitous kinship associations (FKA) was made possible through the recovery at the disaster site of at least one remains for every sought-after victim, and was incorporated into the kinship software. The data from the 13 combined STR loci produced 6 and 23 times fewer FKAs when compared with PP alone and AmpF/STR® Profiler™ (PR) alone, respectively. Identification leads or confirmations of identification were obtained for 218 victims for which DNA reference samples (personal effects and kin) had been submitted. Confirmation of an inferred kinship association was sought through frequency and likelihood calculations, as well as corroborative data from other identification modalities. The use of a simple, yet powerful, automated genotype comparison approach and the use of megaplexes with high power of discrimination (PD) values extended considerably the identification capabilities in the case of the Swissair disaster. The DNA typing identification modality proved to be a valuable component of the large arsenal of identification tools deployed in the aftermath of this disaster.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, mass fatality incidents, mass disaster, software, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, D16S539

Mass fatality incidents (MFI) that lead to severe body fragmentation of victims can represent daunting identification challenges for forensic investigators. In such situations, the level of complexity of the identification process is largely dependent upon the extent of body fragmentation, the number of remains that can be recovered from the disaster site, and the extent of deterioration of the recovered remains. Historically, procedures such as visual identification, match to dental/X-ray/fingerprint records, and personal effects found with the human remains have been at the center of identification efforts for MFIs (1–3), largely due to their reliability, ease of implementation, speediness, and cost effectiveness. However, these identification procedures often rely on unique characteristics (dental features, old surgical scars, tattoos, old fractures, rings, etc.) and therefore depend on the availability and reliability of records documenting these characteristics, and the recoverability of the specific remains bearing the sought-after features. The recovery of all the remains from a disaster site is often difficult to achieve when environmental challenges are encountered at the site (i.e., explosive impact, high temperature fire, air crash over bodies of water, etc.). Consequently, the recovery of remains specific for each victim may be difficult to achieve. In that regard, the unique ability of genotyping to derive identity information regardless of the type of tissue being examined makes DNA technology a valuable tool in MFI victim identification.

In the past decade, STR DNA analysis has become the primary analytical tool of choice for forensic casework applications and paternity testing laboratories. Before these systems became widely available in 1997 as commercial megaplex kits, STRs were generally used for victim identification in situations where the number of victims was often limited (4–7). As such, DNA typing played a key but secondary role in MFI identifications, being used mostly for the regrouping of fragmented remains sharing the same genotype, confirming tentative identifications made by other methods, or being used for identification only when all else failed (8). A paradigm shift occurred in August 1996 with the Spitsbergen air disaster, and was driven by a paucity of available dental and medical records for the victims, which led to the use of DNA typing as the primary method of identification for 139 of the 141 victims (9). The trend toward extensive use of DNA typing in such incidents was to be further emphasized two years later with the next MFI, that of Swissair Flight 111 (10).

On September 2, 1998, Swissair Flight 111, en route from New York City to Geneva, crashed into the Atlantic Ocean off Canada's coastline, taking the lives of all 229 passengers and crew members on board. The crash led to the massive destruction of the McDonnell Douglas MD-11 widebody jetliner and equally severe fragmentation of the bodies of the victims. The extent of devastation and the difficult nature of the recovery operations conducted at an ocean depth of 70 m led to concerns that, for a significant number of victims, the specific remains required for traditional identification methods would never be recovered.

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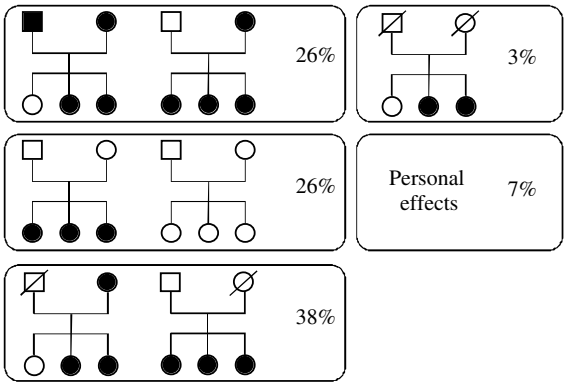


FIG. 1—*Pedigree scenarios encountered for the Swissair MFI. Filled symbols refer to relatives available for reference sampling, open symbols refer to victims, crossed open symbols refer to pre-deceased relatives of the victims. Families are depicted with three offspring to simplify the figure.*

Consequently, an unprecedented DNA typing effort, at the time, was undertaken to process 1277 samples from the crash scene and 397 reference samples obtained from next-of-kin and personal effects.

Through the course of this identification initiative, 228 unique genotypes were derived from the group of tested remains, a number consistent with the flight manifest (there were 229 passengers and crew, but passengers included a pair of identical twins). Because some of the most valuable personal effects for potential use in direct match identifications were lost at sea with the luggage of the victims, parentage analysis was expected to be an important DNA identification modality. Parentage analysis was made more difficult because of four factors. First, complete parentage trios (mother, father and offspring) with only one of the three individuals among the victims, and available known sources of DNA for the other two individuals, were encountered for only 26% of the 229 victims (see Fig. 1). Second, there were 43 families of two to five individuals among the victims, encompassing another 26% of the total number of victims; among these were six families where both parents were traveling with all or nearly all of their children, thereby imposing a requirement for the reconstruction of family pedigrees from within the victims' genotype pool. Third, for 38% of the victims, one of the other two non-victim individuals in the parentage trio was pre-deceased, precluding parentage analysis. Fourth, the necessity to take into consideration the anticipated encounter of core repeat slip mutations in parent:offspring (F1:F2) relationships over such a large number of genotype comparisons added more complexity. In light of these complicating factors, a comparison of each genotype to every other genotype was considered necessary in order to efficiently and reliably delineate relationships among related victims, and between victims and their surviving next-of-kin. As this entailed a very large number of genotype comparisons, software was written to automate the entire pair-wise comparison process, and derive a genetic relatedness index of every genotype to every other genotype. Special provisions for handling core repeat slip mutations were built in. Confirmation of an inferred kinship association was sought through frequency and likelihood calculations, as well as corroborative data from other identification modalities.

We report here on the considerable benefits afforded by this systematic approach to the genotype matching process for the Swissair disaster, and present a critical assessment of the discrimination capability afforded by megaplex-based STR DNA typing kits during this victim identification initiative.

Materials and Methods

Sample Processing

Human remains recovered from the crash site and considered to be probative and capable of yielding DNA results ($n = 1277$; those samples are referred herein as "Q" or questioned samples) were subjected to STR DNA typing analysis. A total of 250 personal effects purported to have belonged to the victims were retrieved from the victims' residences, and 310 reference blood or buccal samples were obtained from family relatives purported to be of direct biological ascent/descent, or spouses of the victims (all referred herein as "K" or known samples). Nearly all family relatives' samples were submitted on FTA™ (Whatman, Clifton, NJ) cards, and these were collected and processed according to the manufacturer's instructions. All other DNA samples were extracted according to standard operational protocols (one-step organic extraction followed by Microcon-100 concentration) and quantitated using the ACES 2.0 chemiluminescence kit (Whatman, Clifton, NJ).

An initial amplification was carried out using the AmpF*l*STR® Profiler Plus™ kit (PP) (Applied Biosystems, Foster City, CA). In situations where complete parentage trios were not available, the samples were also amplified with the AmpF*l*STR® COfiler DNA kit (CO) (Applied Biosystems, Foster City, CA). The combined genotype resulting from the amplification with both PP and CO kits is referred to as the "PPCO" genotype. All amplifications were carried out in strip-capped thin walled 0.2 mL Perkin Elmer MicroAmp™ reaction tubes. The PCR reaction volume for victim and all reference samples not submitted on FTA cards was 25 μL, and submitted to 28 cycles of amplification. Reference samples submitted on FTA cards were amplified directly from 1.2 mm punches in 25 μL reactions, and submitted to 25 cycles of amplification. The cycling conditions were the following: 95°C 11 min, once; 94°C 90 s, 59°C 90 s, 72°C 60 s, for 25 or 28 cycles depending on type of sample; 60°C 30 min, once; 22°C overnight (11). All amplifications were carried out in Perkin-Elmer Gene Amp™ 9600 DNA thermal cyclers.

Amplicons were analyzed on ABD 377 DNA sequencers. For amplicons, a 1.5 μL aliquot of each PCR reaction was diluted in 4.5 μL of loading buffer (2X TBE, 20 mM EDTA, 20 mg/mL blue dextran, 0.5 μL GeneScan 500-ROX (ABD), 9M urea), heat-denatured at 95°C for 2 min and snap-cooled in ice water. An allelic ladder solution was prepared by mixing 0.7 μL of each of the three ladders provided with the kits and 4.5 μL of loading buffer. For both amplicons and allelic ladders, a volume of 1.5 μL was loaded on 0.2 mm thick, 4% 19:1 acrylamide (Bio-Rad)/bis-acrylamide (BRL) 6M urea gels cast on 36 cm well-to-read plates with square-tooth combs. The gels were polymerized by making the solution 0.05% for TEMED and APS, cured for 2 h and pre-run for 30 min at 1000 V. Electrophoresis was carried out at 3000 V for 2.5 h at 51°C in 1X TBE. On each gel, two lanes were reserved for allelic ladder samples.

Independent analysis of each gel file was carried out by two analysts with the GeneScan Analysis v. 2.1/3.1 and Genotyper v. 2.0 softwares. The generated genotype tables from both analysts were checked for concordance before exporting the data into the Kinship Analysis (KA) software. As the vast majority of genotypes derived from remains were complete or nearly complete, remains were regrouped according to genotype. Each group of remains was

considered to represent one discrete victim and the group's consensus genotype was used to query the databases.

Genotype Comparisons

The Kinship Analysis package was written in Visual Basic and performs the following main functions:

(1) searches for perfect matches between Q genotypes and those of personal effects;
(2) for each queried genotype, the software calculates a genetic relatedness index between query and each genotype in the interrogated database;
(3) identifies matching rare alleles and potential ±1 core repeat slip mutations; and
(4) for each queried genotype, the software produces software-annotated score reports displaying the best scores.

A direct match between a Q genotype and the one derived from biological trace evidence recovered from a personal effect purported to have belonged to a victim was a fast and effective means to secure a DNA identification lead. However, the lack of personal effects for numerous victims or the uncertain source attribution of some personal effects made this approach nonapplicable in many situations. The availability of next-of-kin genotypes allowed for so-called "paternity" or "reverse paternity" style analysis, referred herein as parentage analysis, to be attempted. However, the ideal parentage trio in this identification initiative, which ideally includes two known genotypes, could not be assembled in situations where ascendants or descendants expected to provide the known genotypes were themselves pre-deceased or victims in this disaster. Therefore, the only type of genotype comparison scheme that can be used in this context is the pair-wise comparison. This approach permits: (i) direct matches to personal effects, where alleles at nearly all tested loci are expected to match, with the possible exception of a few alleles that may drop out as a consequence of the damaged condition of the questioned sample; (ii) F1:F2 relationships where, according to Mendelian inheritance rules, compared genotypes are expected to share at least one allele at all loci being tested; and (iii) the detection of higher than average sharing of alleles between most siblings, by virtue of their common restricted ancestor allelic background. Consequently, pair-wise comparisons were chosen as the basic comparison tool for the automated part of the process, despite the impact on computing requirements brought about by the large number of comparison events.

For the purpose of ranking samples from high to low genetic relatedness when compared to a queried genotype, the following metrics were compiled for every pair-wise comparison. The number of loci at which at least one allele is shared (herein referred to as the Single Match or "SM" score) between the queried and interrogated genotypes was considered the "Kinship Index" for the purpose of scoring and sorting the interrogated datasets. To complement the SM score, the following scoring tools were also used: the Double Match or "DM" score, representing the number of loci at which both alleles are shared; a Rare Allele score, representing the number of matching rare alleles (rarity being arbitrarily defined as alleles encountered at a frequency of less than 1% in a reference Caucasian population database). For entries with a SM score of 8 out of 9 tested loci for PP, or 12 out of 13 tested loci for PPCO (see Table 1 for listing of loci for the STR kits used in this initiative), when at least one entry allele was found to be at ±1 repeat away in value from that of one of the queried alleles at the locus where a non-match was declared, then the comment box on the score report would be software-annotated to alert the reviewer to the possibility of a core repeat slip mutation.

TABLE 1—*STR loci comprised in AmpF*ℓ*STR® megaplexes and their associated PD* values.*

Megaplex/ Locus	Profiler	Profiler Plus	COfiler	Profiler Plus + COfiler
D3S1358	0.078	0.078	0.078	0.078
vWA	0.065	0.065		0.065
FGA	0.036	0.036		0.036
D8S1179		0.067		0.067
D21S11		0.045		0.045
D18S51		0.030		0.030
D5S818	0.140	0.140		0.140
D13S317	0.074	0.074		0.074
D7S820	0.061	0.061	0.061	0.061
CSF1PO	0.122		0.122	0.122
TH01	0.094		0.094	0.094
TPOX	0.211		0.211	0.211
D16S359			0.103	0.103
Estimated PD	2.8×10^{-10}	1×10^{-11}	1.2×10^{-6}	2.6×10^{-15}
1 in . . .	4×10^{9}	9.5×10^{10}	8.4×10^{5}	3.9×10^{14}

* Power of discrimination values from a U.S. Caucasian population (15).

Each genotype was queried against all other genotypes according to the scoring algorithm specified above, and a score report was generated. Every score report was independently reviewed by two analysts to confirm either a complete match to the genotype derived from a known personal effect, the software-inferred family relationships, or both. For each tentative identification, each proposed family relative's genotype was queried against that of all victims to confirm that the purported relative did not show stronger genetic relatedness to any other victim. The relationships proposed by this triage software were then subjected to standard frequency and likelihood calculations to verify the inferred relationships. An identification by DNA would be considered confirmed when the likelihood of probable relationship exceeded a threshold of 10^6. As identification of victims progressed and the number of unidentified victims decreased, the likelihood threshold was adjusted accordingly. For certain situations, frequency calculations were performed with the help of the STRQuest program (12) and likelihood calculations were performed with a separate in-house software called Trio. In cases where entire families had perished and for which few relatives were available as kinship references, more complex pedigree analysis was performed with the help of the DNAView software (13).

Results

Delineating Kinship

Two types of queries were implemented, both designed to locate, within the interrogated datasets, genotypes consistent with being genetically related to the queried genotype. The first type of query (referred to as type 1) compared each Q genotype to every other Q and K genotype: Q samples were compared to each other as there were 43 families among the victims. The second type of query (referred to as type 2) compared each next-of-kin's genotype to every Q. As each identification lead emerged from type 1 queries, the analysts consulted the type 2 score report of each relative inferred to be related to the victim to verify that no other kinship inferences could be made against another victim not related to the one being queried. Pair-wise comparisons were fully expected to produce

FKAs, but the use of complementary queries facilitated their detection. To increase computing efficiency, genotypes from relatives were segregated into two separate smaller databases according to the gender of the sought-after victim(s), the gender of the queried Q genotype directing which database would be interrogated.

Figure 2 shows a typical score report for a male Q genotype being queried against Q and K genotypes (type 1 query) under the described scoring algorithm. Each report displays the 18 highest scores out of all available Q and K genotypes (459 genotypes in this gender-specific database). The figure demonstrates the usefulness of this reporting format as, for nearly all tested Q samples, true next-of-kin have systematically ranked among the top 18 scores (top 3% of all entries) when sorted under the SM scoring scheme. This allowed for easy examination of the results under one view. Figure 2 shows a parentage situation where every allele of the Q genotype can be accounted for in the genotypes of the two parents. As with any potential kinship inference detected under a type 1 query, the genotypes of the relatives presumed to be linked to the victim were subjected to a type 2 query. As depicted in Fig. 3, this second type of query confirmed that although several other Q samples showed high SM scores in this pair-wise comparison scheme against the three K genotypes being tested, the genotype of Victim A was the only one common to all three relatives, providing a very clear lead to identification. As every allele, including a rare allele, encountered in the victim's genotype could be accounted for in the genotypes of the purported parents, and a complete match across all loci to the genotype derived from a toothbrush was observed, this data confirmed the identification for this victim.

Rare Alleles

In order to increase the sensitivity of the triage tool, additional weight was given to matching rare alleles. Even though the victims and their surviving relatives were from 21 different nationalities, approximately 90% of victims were Caucasian. Although allelic frequencies can vary slightly from one ethnic group to the next and, as such, a rare allele in one group can prove to be common in another group, the scoring of rare alleles, as defined by frequencies observed in a reference Caucasian population, proved informative in numerous instances. While occurrences of rare allele detection involved one or, more rarely, two alleles in the same scoring event, there was one exception where three rare alleles were encountered in the non-Caucasian parentage trio featured in Fig. 4.

Fortuitous Kinship Associations (FKA)

Scores of SM9 (PP)/SM13 (PPCO), or SM8 (PP)/SM12 (PPCO) plus one core repeat slip mutation (referred herein as "SM8 + 1", "SM12 + 1") were considered suggestive of a potential F1:F2 relationship. Under these conditions, assuming an average 50% chance (approximated from the PP allele frequency tables) for unrelated individuals to randomly match at least one allele at any given random locus, the odds of encountering a FKA (SM9 or SM8 + 1 for PP) between any two randomly selected genotypes were originally estimated at 1 in 300–500 (0.5^8–0.5^9). Considering the average number of genotypes being compared for each query (average = 450), one to two FKAs were expected to be encountered for every queried genotype. An example of such a FKA is shown in Fig. 2 where Victim B's genotype did score as well as the true parents of Victim A. Considering the high scores encountered with all of Victim A's relatives, and that Victim A's genotype was entirely accounted for by his two parents in parentage trio analysis, Victim B's score did not challenge the validity of the inferred kinship for family A. However, in situations where only one relative is available as a biological reference for a victim, the presence of such FKAs in a score report can make the kinship more difficult to infer if the FKAs cannot be reliably excluded on the basis of much stronger and extensive ties (i.e., parentage trio) to other victims.

As systematic exclusion of FKAs under our selected comparison scheme can be more efficiently carried out with a closed population (i.e., number of encountered unique genotypes = number of victims on flight manifest), measures to increase the chances of securing a closed population were implemented. One such measure was the large scale processing of Q samples, which developed the expected 228 unique genotypes (Fig. 5). No other unique genotypes were encountered during the processing of subsequent remains.

An average of two entries per score report were expected to be considered as possible FKAs. This number was at times largely exceeded in practice. An increased number of FKAs was generally observed when the queried genotype comprised numerous alleles commonly encountered in the general population. The capability to exclude FKAs proved very valuable in these instances. In one extreme case, the victim was traveling alone, and no next-of-kin could be found to provide reference samples. The personal effects submitted for this victim provided a way to attempt a DNA identification. The victim's genotype (Victim AH), showed a complete match at all tested loci to that derived from a personal effect. However, 38 potential FKAs (eight SM9 and 30 SM8 + 1) were observed in the score report when queried against all Q and K genotypes (data not shown). In this situation, 12 of the 18 alleles in the victim's heterozygous genotype were the most frequently encountered alleles in their respective locus class, as estimated in a random sample of the Canadian Caucasian population. All FKAs encountered in Victim AH's report were excluded after review of the type 2 report for each of the 38 FKAs. When this victim's genotype derived from a personal effect was tested against only those of the victims, a total of six SM9 and 11 SM8 + 1 were encountered (Fig. 6).

Personal Effects

A total of 250 personal effects, for the most part unwashed undergarments/sleepwear and personal hygiene items such as hairbrushes, toothbrushes, and razors, were recovered by relatives or local authorities from the homes of the victims. Due to the large volume of questioned samples queued for wet bench analysis, processing of personal effects was confined to those purported to have belonged to victims for which few, if any, reference samples from relatives were available. A total of 47 genotypes derived from personal effects were eventually used to confirm the identity of many victims. Establishing identity of a victim with a personal effect generally proved straightforward, as shown in the situation featured in Fig. 2, where a complete match across all tested loci between the genotype derived from the Q sample and that of a toothbrush was noted. However, 10% of submitted personal effects, typically misattributed personal hygiene items, produced a genotype other than that of the expected victim, most often the spouse's. This was anticipated in situations where couples were among the victims. Source attribution of personal hygiene items like a toothbrush or a razor can prove difficult to establish when relatives recovering the items do not share the same household as the victim. In many situations, gender discriminating data (amelogenin locus) assisted in dissipating confusion over source attribution. Consequently, confirmation of a complete match to a personal effect was sought through consistent kinship analysis data, whenever possible.

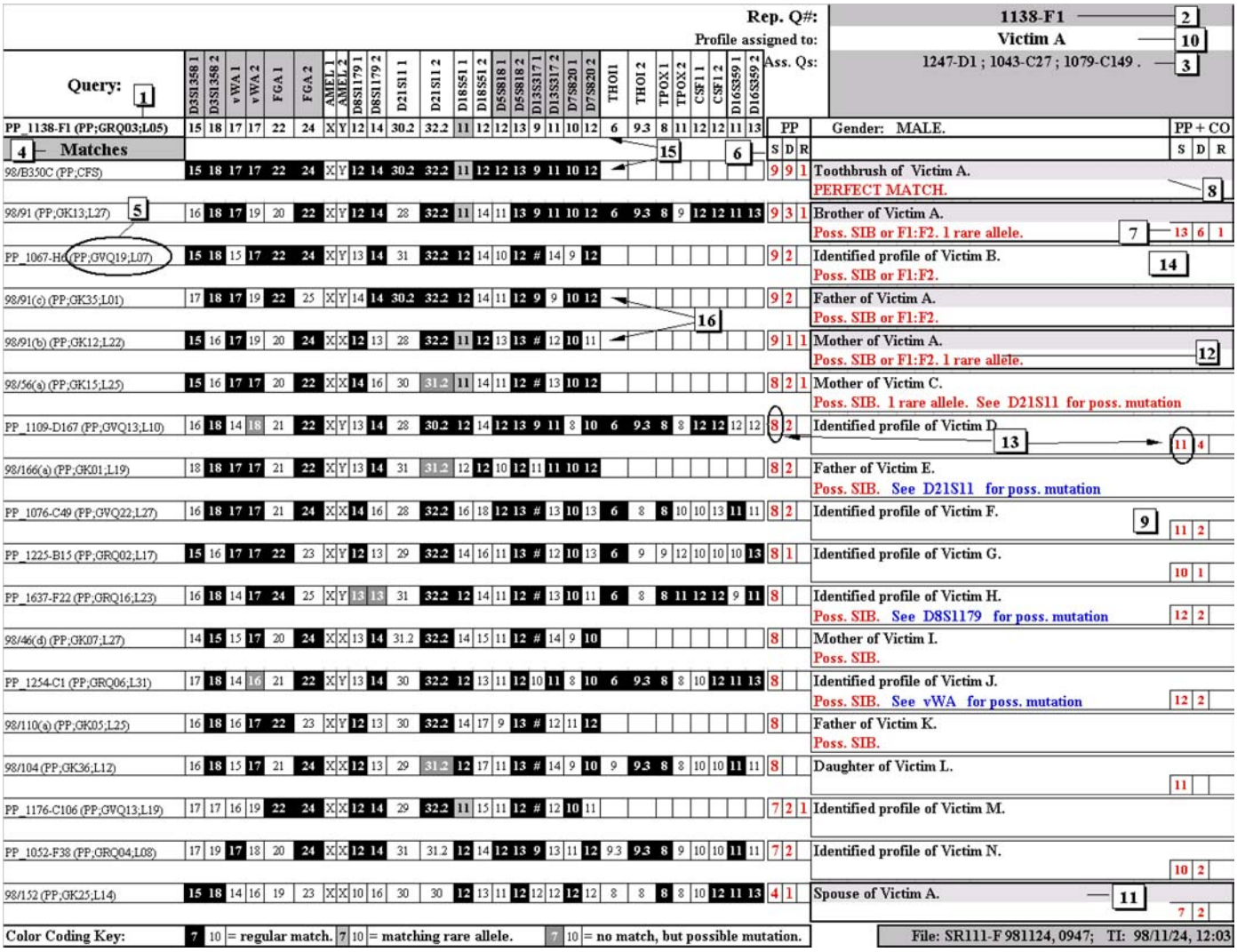


FIG. 2—*Example of a type 1 score report. (In order to protect the privacy of the individuals involved, sample names and identification numbers have been changed/anonymized in all figures displaying genotypes throughout this manuscript.) This figure is an example of a typical score report for a type 1 query, where a Q genotype is queried and scored against all Q and K genotypes. One report was generated for each of the 228 unique victim genotypes. In parallel, a similar report was generated for each of the 357 reference (next-of-kin and personal effects) genotypes. (1) Queried genotype. (2) Q samples sharing the same genotype are regrouped and one Q sample is selected as a representative of the sub-grouping (Rep. Q) for querying the database; the remaining Qs in the group are referred to as Associated Qs. (3) Associated Q numbers are automatically retrieved from the main Q database. (4) Scored genotypes, against queried Q. The software scores the queried Q genotype against all K genotypes for which a victim of the same gender is sought, as well as all Qs. (5) For sample tracking purposes, each entry is electronically amended to include an identification string containing the megaplex name, submitting RCMP laboratory, known or questioned gel, batch/gel and lane numbers. (6) Entries are sorted according to Single Match (loci at which only one allele was found to match), Double Match (loci at which both alleles were found to match) and Rare Allele (loci at which a rare allele was found to match) scores. Amelogenin alleles are not scored. Only the 18 highest scores (out of an average of 450) appear on a report. To facilitate interpretation, the software color codes all cells for matches according to the Color Coding Key appearing at the bottom of the report. (7) When CO data are available for both queried and a scored genotype, then the combined PPCO scores are displayed. (8) Sample information is displayed to facilitate the inference of kinship. Information provided by family relatives is entered into the system. Qs show up as "Unidentified Q" until identification has been made. (9/10) Once identification is made on a Q sample, the victim's name automatically appears with its associated genotype. (11) If "Profile assigned to" box displays a victim's name (see item #10 above), then the program makes room at the bottom of scoring report to display all other entries of the same family that did not score in the highest 18 scores. The software also highlights all entries from the said family with a background shade. (12) Software-generated conclusion statements are generated only for SM8/SM12 or SM9/SM13 scores. Conclusion statements take into account SM scores and the presence of the rare alleles. With SM8/SM12 scores, the software assesses the non-matching locus for the possibility of a one core repeat slip mutation. If found, the allele in question is flagged and the conclusion statement includes a notification. (13) When CO data are present, the PPCO conclusion supersedes the PP conclusion. In this case, an SM8 score with Profiler Plus linked to a possible mutation at vWA, although flagged on the genotype, is not considered in the conclusion statement because the score did not reach the SM12 threshold with the addition of CO data. Therefore, no conclusion statement was generated. (14) Third entry into this list is an example of a fortuitous kinship association (FKA). (15) Identification with a perfect match to a personal effect. (16) Typical parentage situation where all alleles in the genotype of the victim can be accounted for in the genotype of the parents.*

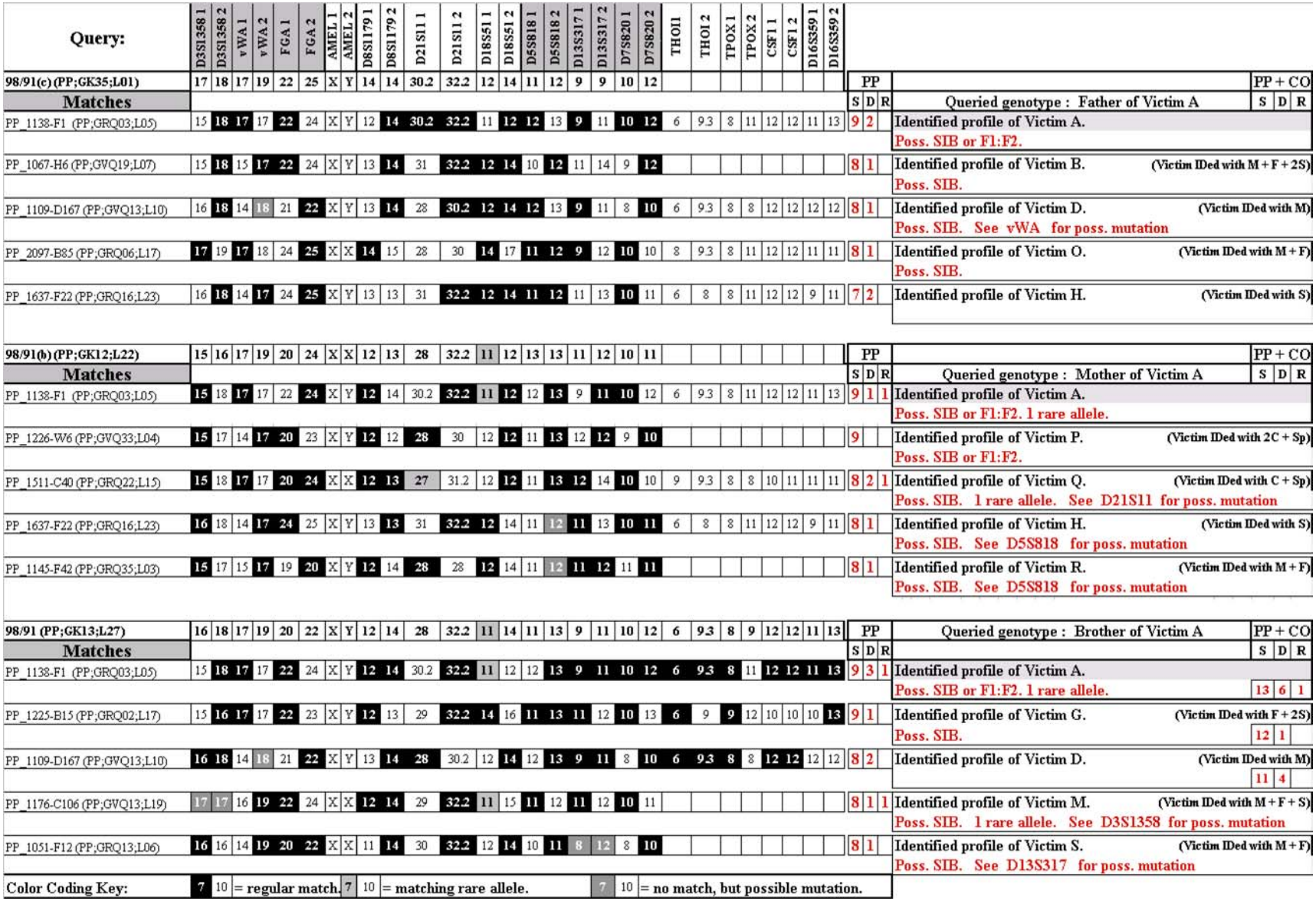


FIG. 3—*Examples of a type 2 score report. In such reports, next-of-kin genotypes that triggered an identification lead in a Q report are scored against all Questioned genotypes. One report was generated for each of the 310 next-of-kin genotypes. This figure specifically displays panels from the type 2 reports produced for the three relatives purported to be the next-of-kin of Victim A in Fig. 2. The panels display the highest scores encountered for these three relatives when their genotypes are scored exclusively against the genotypes of the victims. The reasons why scoring genotypes have been excluded as possible contenders are displayed in the upper right corner of the comment boxes, according to the following legend: M = mother; F = father; C = children; S = sibling; Sp = spouse; PE = personal effect.*

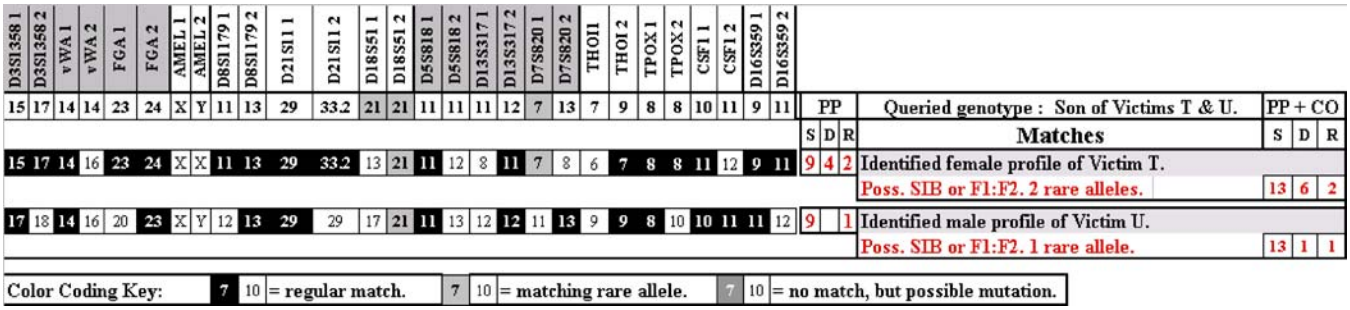


FIG. 4—*Usefulness of rare alleles. This figure features an excerpt of an unusual case where three rare alleles (D18S51 allele 21 twice; D7S820 allele 7), two of them homozygous at the same locus, were encountered in a next-of-kin whose genotype was used to identify both of his parents among the victims. These two entries scored first and second on a total of 228. The third entry scored as SM7, DM1.*

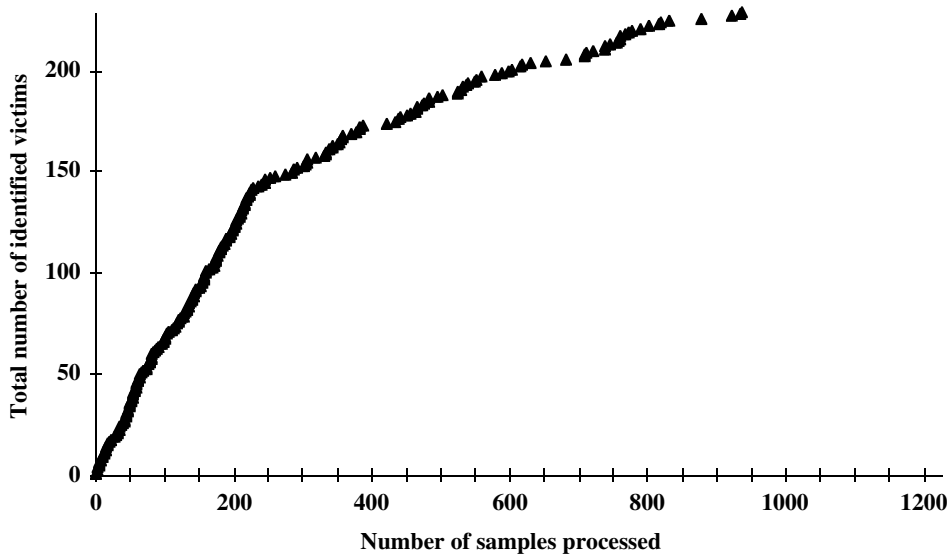


FIG. 5—*The progression of the Swissair identification initiative. This figure shows the chronology of identification. Most of the first 200 remains originated from separate victims. Beyond that point, according to the principle of diminishing returns, most of the remains collected matched back to victims for whom at least one remains had been recovered. Some 300 remains were DNA typed in order to secure the last five unique genotypes.*

Core Repeat Slip Mutations

Core repeat slip mutations are the means by which variation in STR length is created. Therefore, such mutations are expected to be detected at a low frequency when examining the genotypes from individuals in F1:F2 relationships. The software was configured to detect potential core repeat mutations, as shown in Fig. 2. A total of six mutations were noted in confirmed F1:F2 relationships, and examples are displayed in Fig. 7 (complete list can be found in Table 2). In one situation (Victim V), a mutation was detected at the FGA locus between the genotype of the victim and that of his father. The genotype of Victim V's sister also had a mutation when compared to that of her father's genotype, but at a different locus, vWA. This demonstrated the rare circumstance of two different somatic mutational events occurring in two different children of the same parents. Parentage analysis allowed to unequivocally establish kinship of all four individuals, despite the observed mutations.

When several relatives were available as references for a victim, the encounter of a core repeat slip mutation did not present interpretation difficulties. In that respect, the second example of mutation in Fig. 7 demonstrates a more complicated situation where the mother was the only surviving relative for the victim. When querying Victim Y's genotype against all Qs and Ks, no SM9 scores were observed, but 8 SM8 + 1 scores were detected, including the putative mother's genotype. Conversely, when querying the putative mother's genotype against those of all Qs, only 2 SM8 + 1 scores were detected, including Victim Y's genotype. All FKAs for both queries were easily excluded as they showed significantly stronger relatedness (i.e., part of parentage trios, match to a personal effect) with other victims and their relatives. Considering the closed population situation attained in this MFI investigation and the absence of any other unassigned genotypes showing significant kinship indices, a DNA typing identification lead for Victim Y was considered valid, even in the absence of a parentage trio and the presence of a core repeat mutation. The DNA typing result for Victim Y was confirmed by independent dental and X-ray identifications.

TABLE 2—*Mutational events encountered in F1:F2 relationships.*

Case No.	Citizenship	STR Locus	F1 Allele*	F2 Allele	Mutational Event
1	France	vWA	20 (F)	19	Contraction
2	France	D18S51	14 (M)	15	Expansion
3	U.S.A.	FGA	24 (F)	25	Expansion
4	U.S.A.	vWA	17 (F)	18	Expansion
5	U.S.A./ Switzerland	D21S11	29 or 31 (M)	30	Expansion or Contraction
6	U.K.	D13S317	14 (F)	13	Contraction

* F = father; M = mother.

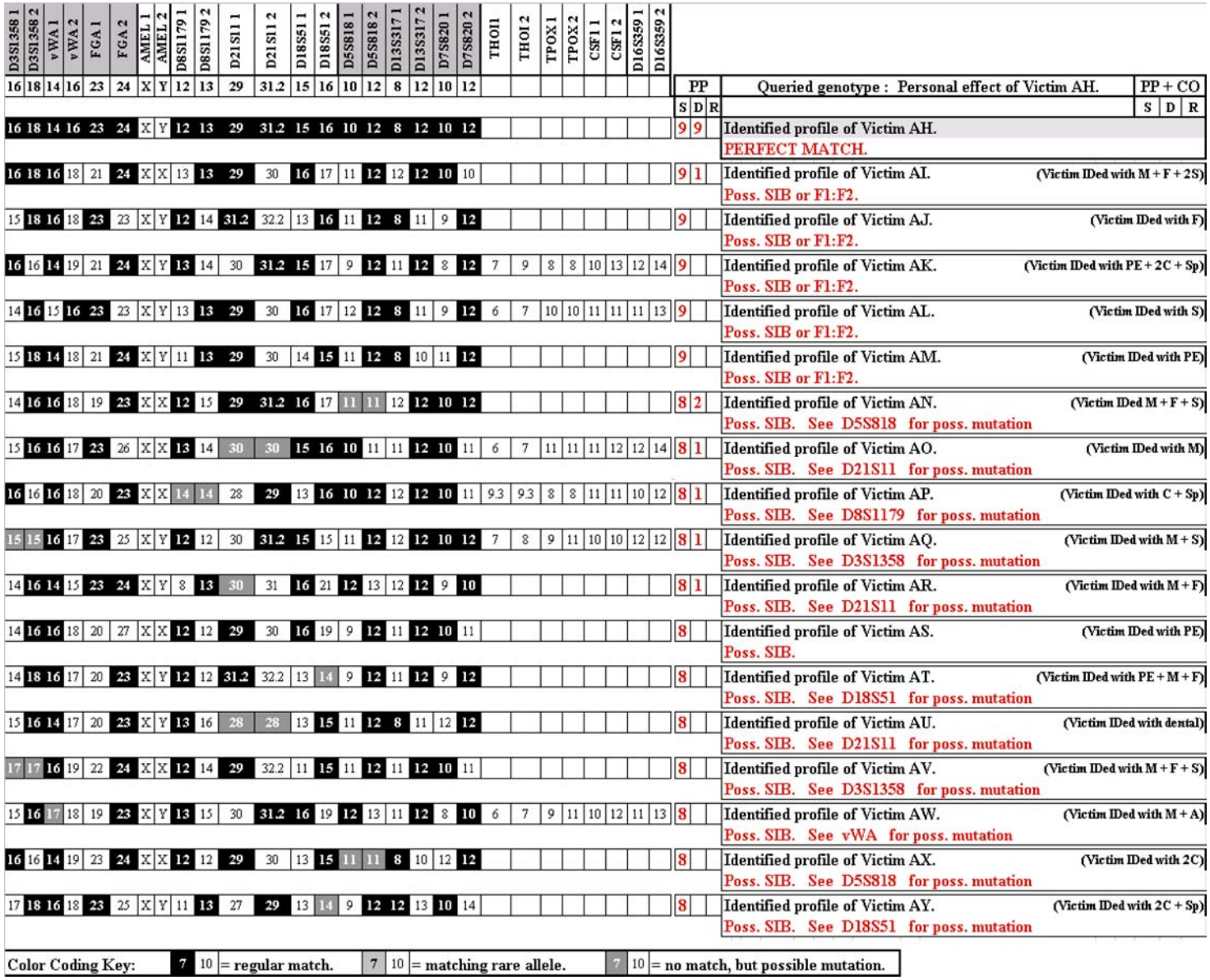


FIG. 6—*Fortuitous kinship associations (FKA). This figure features the type 2 report for a personal effect genotype showing the greatest number of FKAs encountered for a single queried genotype. The perfect match to a K genotype derived from a toothbrush was sufficient for identification, but a total of 17 potential F1:F2 relationships (6 SM9s + 11 SM8 + 1; 16 are displayed here) were encountered when tested against genotypes of other victims. When this genotype was tested against all victims and family relatives for which a male relative was being sought, 38 potential F1:F2 relationships (8 SM9s and 30 SM8 + 1) were encountered (data not shown). Displayed at the right-hand side of the sample identification box is the reason why the associated score was excluded as a contender, usually because of a stronger genetic relatedness to another Questioned genotype (M = mother; F = father; C = children; S = sibling; Sp = spouse; A = aunt; PE = personal effect). Victim AL was excluded on the basis of a SM12, DM5 score (17 matched alleles out of a possible 26) against a sibling, for PPCO.*

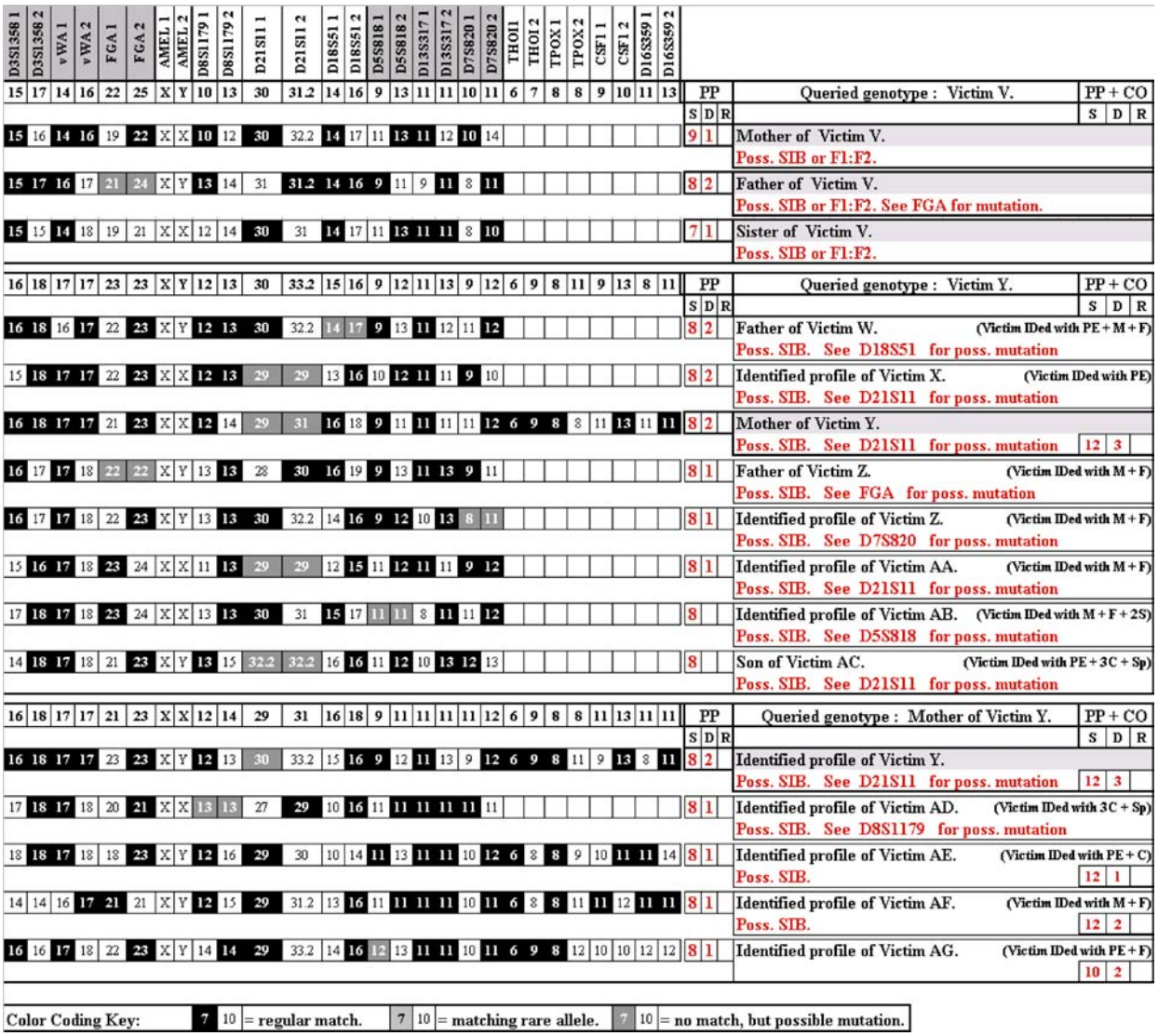


FIG. 7—*Core repeat slip mutations. The top panel of this figure features a situation where the Victim V along with his sibling both displayed core repeat slip mutations but in different loci when their genotypes were compared to their father's. Scores relevant to the issue at hand are displayed. The following two panels refer to a Victim Y who displayed a single repeat slip mutation upon comparison of his genotype with his mother's, his only living relative. There were no SM9 scores in the middle panel and only SM8 + 1 scores against the queried victim's genotype are displayed. The reasons why other scoring genotypes have been excluded as possible contenders are displayed in the right corner, according to the following legend: M = mother; F = father; C = children; S = sibling; Sp = spouse; PE = personal effect. The third panel displays the four highest scoring genotypes against the queried next-of-kin genotype.*

High Genetic Relatedness

High genetic relatedness was encountered in a few families. In the first of two cases presented in Fig. 8, an impressive sharing of alleles is noted among the children of this family, which proved very useful for the identification process. This high level of relatedness among siblings could find an explanation in the fact that the father's genotype featured five loci in an homozygous state, and that at least one allele was found to match at seven out of nine loci when his genotype is compared with that of his spouse. A more extensive matching of alleles was observed with Victim BA in Fig. 8 where the PPCO combined genotype of the victim matched at 23 out of 26 alleles of her sister's genotype.

High genetic relatedness had an unforeseen impact on the parentage analysis result shown in Fig. 9 in the case of two spouses among the victims who have in common nine alleles out of 18 in their PP genotypes. Both parents were available as references for the female spouse, only the mother was available for the male spouse. As shown in Fig. 9, after a first round of amplification with PP, parentage analysis inferred the parent status for the father of female Victim BB, but could not exclude this same individual from being considered as a potential father to his son-in-law, Victim BC. At D13S317, any of the two potential parents could have contributed allele 11, while the other parent would have contributed allele 12 through a core repeat slip mutation. Additional loci data were required to clarify this situation; the samples were subjected to a second amplification with COfiler. With the resulting PPCO data, the father of Victim BB showed a SM13 score against his daughter's genotype, and a SM10 score against his son-in-law, which excluded him as the father of Victim BC. This situation where nine STR loci were insufficient to exclude two unrelated individuals as potential parents in a parentage trio was unique in this identification initiative. However, it demonstrates that, given a large enough number of pair-wise comparisons (71,490 for type 1 queries in this initiative),

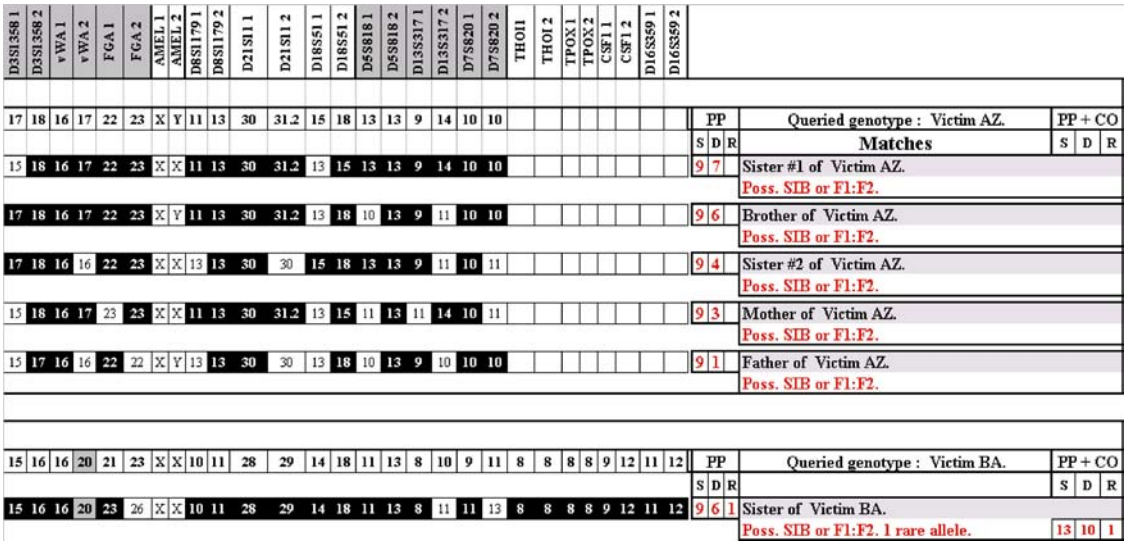


FIG. 8—*Genetic relatedness. This figure features the score reports of two victims for whom very strong genetic relatedness with their next-of-kin was observed.*

these fortuitous situations involving unrelated individuals can arise. It appears reasonable to postulate that a fortuitous parentage trio involving a couple for whom an offspring is searched would be much rarer. In order to detect such events within this identification initiative, samples were reprocessed with the COfiler system whenever any inconsistency or possible ambiguity was encountered between the reported next-of-kin relationship to the victim and the software-inferred relationship, or when the likelihood of probable relationship fell under the prescribed threshold.

Linkage to Second Degree Relatives

Genetic relatedness could also be established with more distantly related individuals. In the first of two cases featured in Fig. 10, four second degree relatives (defined as other than siblings, parents and offspring of the victims), two from each ancestry line, ranked in the top 3% on Kinship Index values against the queried victim, the victim being one member of a family of four victims aboard the aircraft. Similar results were recorded when the victim's sister, also a victim, was tested against the database (data not shown). The identification lead was supported by confirmation of parentage trios involving both parents among the victims and each of their presumed children. Even without any reference samples from relatives or personal effect, the combination of the score reports for all four members of this family clearly suggested a family unit for these individuals, identifying which individuals were F1s and F2s, confirming two parentage trios. This effectively reduced the number of possible family names to very few, considering the known compatible family pedigrees reported to be missing. Most family clusters among the victims could be detected prior to testing against any K genotypes. In the second case depicted in Fig. 10, again four second degree relatives of the victim, two from each ancestry line, ranked in the top 3% on Kinship Index values against the queried victim. In this instance, the identification lead was later confirmed by standard parentage trio analysis when remains of both parents of the victim were recovered.

Discrimination Capability of STR Megaplexes

Features designed to compile performance statistics were built into the software to systematically collect data on all genotype comparisons, a total of 71,490 for PP alone, for type 1 queries. In addition, as 15,363 of these genotype comparisons involved samples for which both PP and CO data were available, a comparative assessment of the performance of the different loci combinations used in the ABI megaplexes was carried out. Since the 13-loci data encompassed the loci comprised in the PR kit, it was possible to simulate the performance of that kit as well, as it pertained to its power of discrimination. Considering the reported PD values for the available megaplexes (see Table 1), the purpose of this comparison was to assess: (i) the gains that a database-wide use of CO as a complement to PP would have afforded, and (ii) the impact on kinship analysis interpretation if a less discriminating megaplex like PR had been selected as the first line system instead of PP. The chosen parameter by which performance was assessed was the SM score, which was found to be more useful for kinship analysis than a total score encompassing all matching alleles. Figure 11 presents the SM scoring data in both graph and tabular form. A normal distribution of SM scores was observed for all tested and simulated megaplexes. The presence of family clusters in this dataset did not affect the score distribution for any megaplex as the number of genotype comparisons involving truly related individuals represented only 0.6% of all comparisons carried out. One also notes that the PP distribution curve for the full database (samples genotyped with PP), although on a different scale, nearly superimposes on the PP curve for the restricted database (samples genotyped with both PP and CO), confirming that the sampling size of the restricted database is sufficiently large to be representative.

For score interpretation as it relates to kinship analysis, scores of SM9 for the 9-loci megaplexes, or SM13 for the 13-loci were of particular importance because of their significance in inferring possible F1: F2 relationships. SM9 or SM13 scores were often encountered in sibling relationships as well. Any SM9 or SM13 scores stemming from genotype comparisons involving non-related individuals are considered chance occurrences and, for the purpose of this kinship analysis protocol, are referred to as FKAs. The observed frequencies of such FKAs were calculated (Table 3) for the different megaplexes and demonstrated that, as expected, the higher the PD value, the fewer FKAs are encountered. As normal distributions of SM score data points were observed with all megaplexes (Fig. 11) and were not affected by the presence of family clusters, the average SM values for each megaplex were used

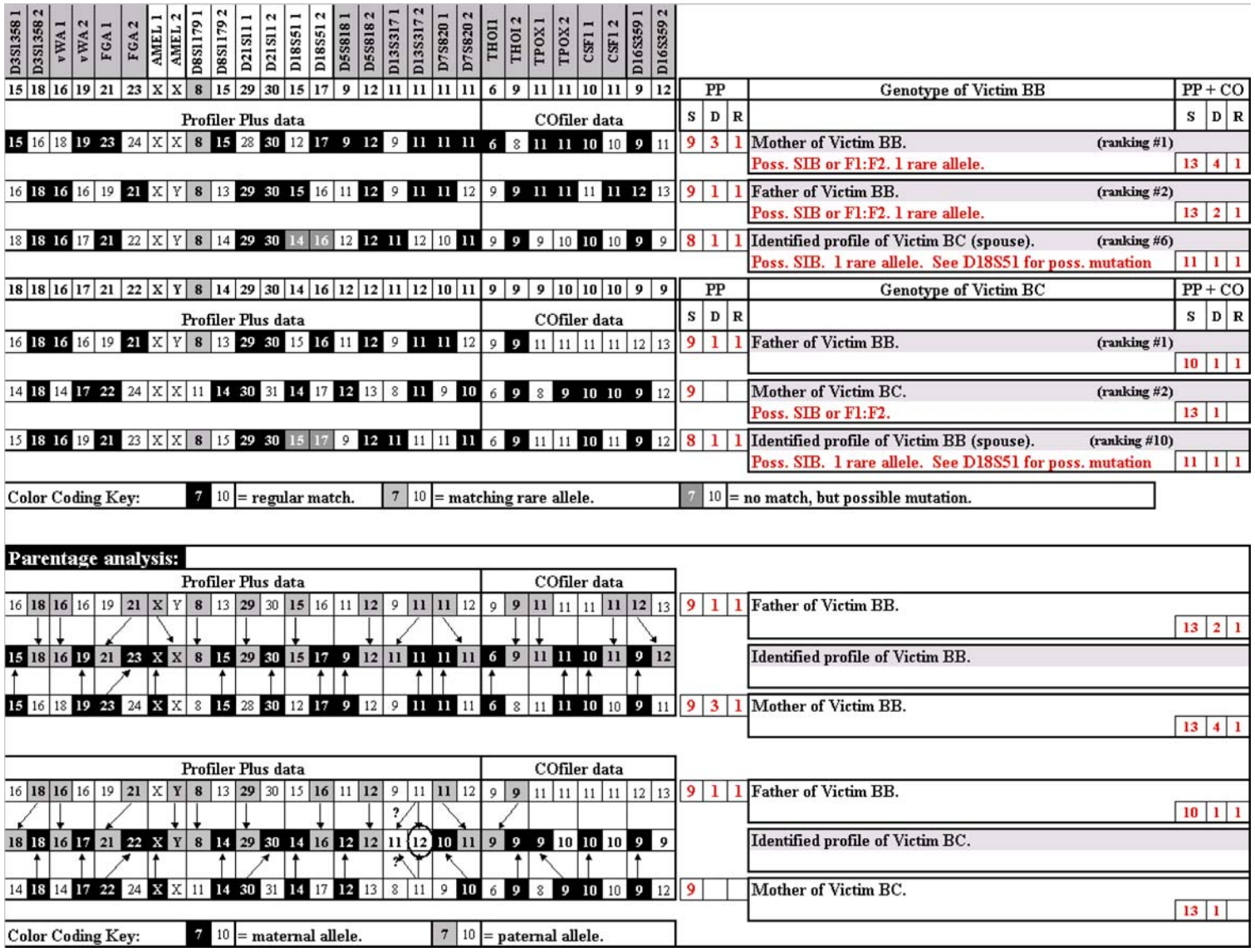


FIG. 9—*An unusual parentage analysis situation. Before the segregation of relatives according to the gender of the sought after victim was implemented, an unusual parentage situation was encountered. Victims BB and BC were listed as being married on the flight manifest. Both spouses unexpectedly ranked in the top 18 scores when their genotypes were tested against each other. In addition, the father of Victim BB ranked #1 against the genotype of his son-in-law. The parentage analysis of Victim BB confirmed the F1:F2 relationship to the expected relatives. When considering only PP data, the parentage analysis for Victim BC could account for every allele in the victim's genotype with one core repeat slip mutation, and could not eliminate the father-in-law as a father for Victim BC. The samples were reprocessed with CO to resolve this enigma. With a full 13-loci complement, four core repeat slip mutations would have had to be considered for the inclusion of father of Victim BB as a potential father to Victim BC. On this basis, the father of Victim BB was excluded as a potential father to Victim BC.*

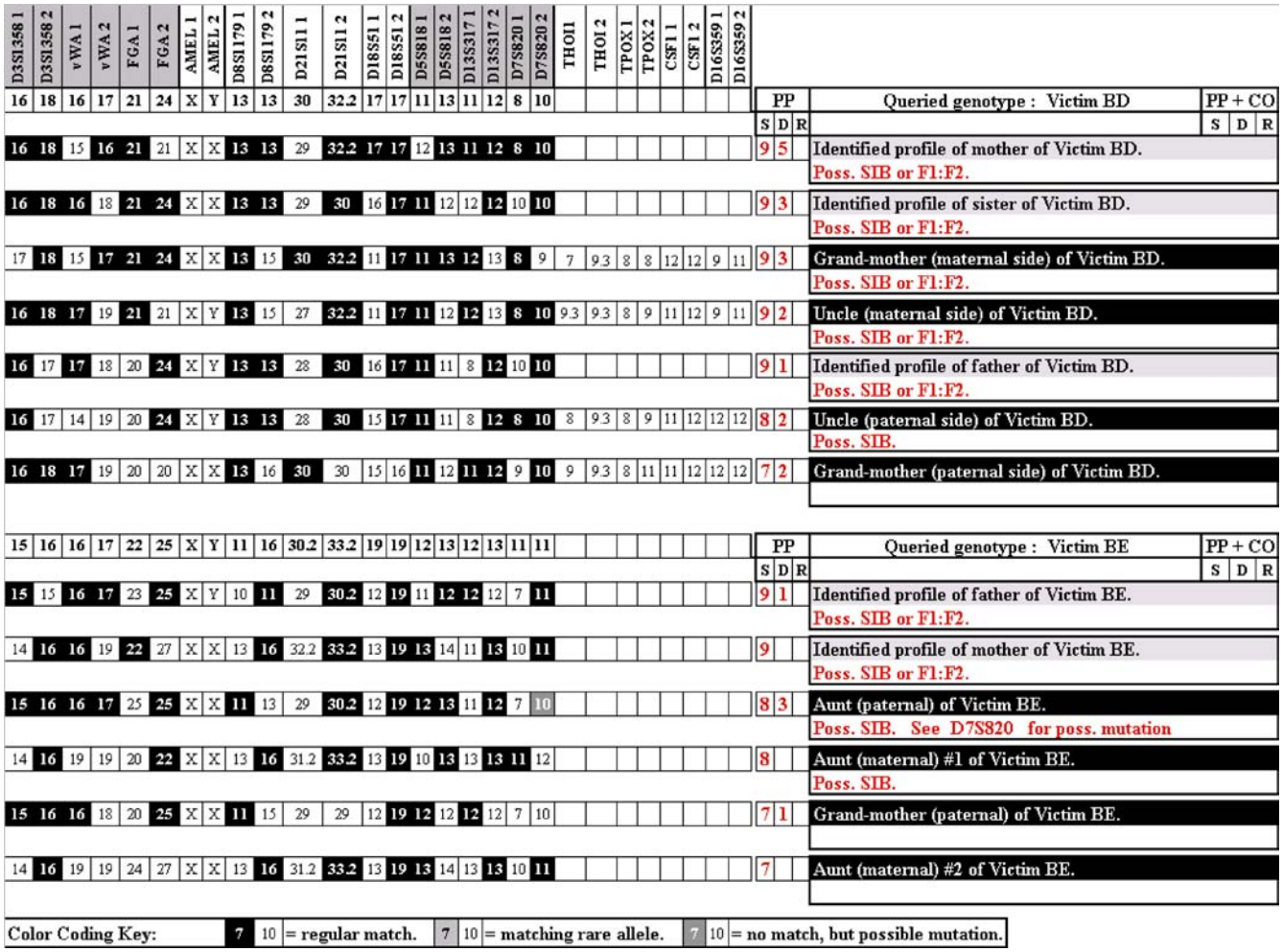


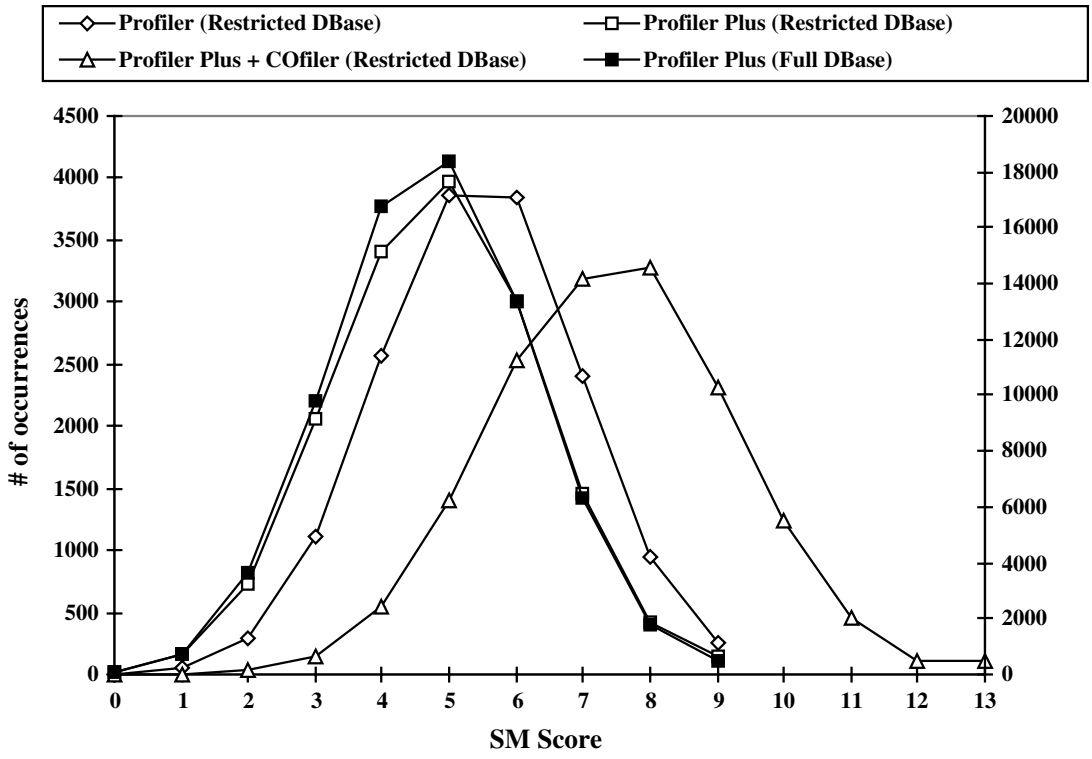
FIG. 10—*Establishing relatedness through distant relatives. This figure features the score reports of two victims for whom genetic relatedness to next-of-kin is apparent, even with distantly related living relatives. These informative living distant relatives are highlighted with a black background.*

to calculate expected FKA frequencies (Table 4) and these were shown to be in good agreement with the observed values. However, the observed number of FKAs for the PR kit exceeded the expected value, most likely reflecting the presence in that kit of the lesser discriminating CTT (CSF1PO, TH01, TPOX) loci. The PPCO combined genotype approach produced 6 and 23 times fewer FKAs when compared with PP alone and PR alone respectively (Table 4). Observed values for the restricted database were extrapolated to predict the performance of PR as well as of PPCO, if these combinations had been used instead of PP alone on the entire sample set. Over 500 additional SM9 scores, an average of two per queried genotype, would have required demonstration of stronger genetic relatedness to other victims before being declared FKAs if PR had been used instead of PP.

Single core repeat slip mutations occur occasionally upon STR allele transmission from parent to offspring and are encountered at a low frequency in the population. Single repeat mutations are much more frequent than slips of multiple repeats (14). In order to facilitate the identification of F1:F2 relationships involving such mutational events, the kinship analysis program was set to flag any entry with a score of SM8 for PR or PP, or SM12 for PPCO for which the non-matching locus had at least one allele showing a difference of exactly one repeat with any of the queried genotype's alleles for the same locus. As shown in Table 5, the PPCO combined genotypes produced four and nine times fewer SM8/SM12 scores compatible with a single core repeat slip mutation scenario when compared to PP alone or PR alone, respectively. When extrapolating to predict the performance of PR as well as of PPCO, if these had been used instead of PP alone on the entire sample set, close to 2000 additional SM8/SM12 scores compatible with a repeat slip mutation scenario, an average of nine per queried genotype, would have required demonstration of stronger genetic relatedness to other victims before being declared non-F1:F2s, if PR had been used instead of PP.

Discussion

DNA typing enables identity information to be derived from any type of tissue. This makes it an ideal technology for the identification of victims of high body fragmentation MFIs. Assuming a full nine or thirteen STR loci genotype can be derived from both a personal effect and a Q sample involved in a comparison, a direct match can be considered as substantial evidence of identity. However, problems with source attribution of personal effects can be encountered. Therefore, working on the assumption that source attribution problems will affect only a minority of submitted samples, it is good practice to process multiple personal effects for each victim, whenever possible. It is preferable still to confirm source attribution by parentage analysis of the genotype derived from the personal effect with the genotypes derived from family relatives. In



	SM scores														
Restricted database	0	1	2	3	4	5	6	7	**8**	**9**	10	11	**12**	**13**	Total
Profiler	1	59	297	1114	2565	3863	3842	2404	**955**	**263**					15363
Profiler Plus	11	167	727	2065	3413	3965	2999	1454	**417**	**145**					15363
Profiler Plus + COfiler	0	5	33	149	550	1400	2534	3191	3279	2319	1234	453	**111**	**105**	15363
Full database															
Profiler Plus	55	748	3638	9847	16754	18435	13407	6351	**1745**	**510**					71490
Profiler Plus (- related)	55	745	3623	9833	16712	18395	13371	6313	**1698**	**177**					70922

FIG. 11—*Scoring performance of tested megaplexes. The graph shows the distribution of SM scores tabulated for every comparison carried out in type 1 queries. The PR data comes from a simulation, as the actual kit was not used in sample analysis. The Qs vs. Qs scoring totals (not specifically shown) were halved to account for database redundancy (any given comparison being computed twice, once as A vs. B, then as B vs. A). The secondary axis at right pertains to the PP full databases data. Tabulated scores representing potential F1:F2 relationships (SM9 or SM13) and possible one core repeat slip mutations (SM8 + 1, or SM12 + 1) are highlighted. The restricted database dataset refers to the subgroup of samples for which both PP and CO amplification reactions were carried out. The full database dataset refers to samples (all) for which PP was run. The "PP (minus related)" row is the PP full database data from which all comparisons involving related individuals were removed. This last dataset was not plotted on the graph as the curve is indistinguishable from that of the full database, showing only minor differences at SM8 and SM9.*

addition to corroborating evidence derived from personal effects, parentage analysis can be a valuable tool on its own. However, parentage analysis infers a relationship, not an identity. An inferred relationship, especially through a parentage trio, often translated into an identification when no other Q genotype within the MFI population challenged the conclusion. In the case of the 43 families aboard Swissair Flight 111, numerous siblings were present, and these could often be matched back to their family identity through parentage analysis or familial relationships with good probability of association. In the case of same gender siblings, the final identification would rely on the availability of a genotype derived from a personal effect or other identification information (dental, X-ray, etc. . .) on at least one of the two victims.

Parentage analysis has proven its usefulness in numerous disasters in the past (2,4–8) as it was for the Swissair MFI. In this identification initiative, the limited number of probative personal effects, lack of living relatives (parents in particular) for older victims, and the numerous families among the victims created an extremely challenging situation for the DNA typing aspect of the victim identification initiative. In order to rapidly and efficiently infer associations between genotypes derived from human remains recovered from the crash site and those derived from personal effects and relatives, a pair-wise genotype comparison software was written.

In order to optimize the chances of contributing reliable DNA identification data for as many victims as possible, the following approach was taken for the DNA typing portion of this MFI. First, genotypes were derived from as many remains as possible to increase the odds that all 228 victims' genotypes would be encountered. Second, family relatives were solicited to provide reference blood or buccal samples for kinship analysis. Third, family relatives or authorities were requested to retrieve personal effects from the homes of the victims to be used for DNA typing of residual biological traces. Fourth, in order to enable the efficient exclusion of FKAs and increase the chances of making identifications for victims for whom only a single first-degree relative, or one or several second-degree relatives, was available, every genotype was compared with every other genotype. Fifth, corroborative

TABLE 3—*Calculation of fortuitous kinship association (FKA) occurrence rate.*

Megaplex	Total No. of SM9/SM13 in Database[b]	No. of SM9/SM13 Matched to True Relatives[c]	No. of FKAs
	RESTRICTED DATABASE[a]		
Profiler	263	101	162
Profiler Plus	145	100	45
Profiler Plus + COfiler	105	98	7
	FULL DATABASE[a]		
Profiler (estimated)[d]	1029	333	696
Profiler Plus (observed)	510	333	177
Profiler Plus + COfiler (estimated)[c]	363	333	30

[a] Restricted Database: samples processed with PPCO; Full Database: samples processed with PP only.
[b] Except for the estimates, values were derived from Fig. 11.
[c] Represents the number of SM9/SM13 scores where kinship has been officially confirmed between Q and K genotypes, the remainder being considered FKAs (last column). Kinship in this setting encompasses both F1:F2 and sibling relationships.
[d] For PR and PPCO, estimates are an average of the following two values: (1) values obtained with the restricted database were multiplied by 4.65 (= 71,490/15,363 (number of pair-wise comparisons in full database/number of pair-wise comparisons in restricted database)); and (2) values obtained with the restricted database were multiplied by 3.93 (= 177/45; (number of observed FKAs for PP in full database/number of observed FKAs for PP in restricted database)).

TABLE 5—*Calculation of potential core repeat slip mutation occurrence rate.*

Megaplex	Total No. of SM8/SM12 Scores in Database[b]	No. of SM8/SM12 Matched to True Relatives[c]	No. of Potential Core Repeat Slip Mutations
	RESTRICTED DATABASE[a]		
Profiler	955	13	751
Profiler Plus	417	12	320
Profiler Plus + COfiler	111	8	87
	FULL DATABASE[a]		
Profiler (estimated)[d]	4220		3269
Profiler Plus (observed)	1745	45	1297
Profiler Plus + COfiler (estimated)[d]	491		379

[a] Restricted Database: samples processed with PPCO; Full Database: samples processed with PP only.
[b] Derived from Fig. 11.
[c] Represents the number of said scores where kinship has been confirmed between the Q and K genotypes. Kinship in this setting encompasses both F1:F2 and sibling relationships.
[d] For PR and PPCO, estimates are an average of the following two values: (1) values obtained with the restricted database were multiplied by 4.65 (= 71,490/15,363 (number of pair-wise comparisons in full database/number of pair-wise comparisons in restricted database)); and (2) values obtained with the restricted database were multiplied by 4.05 (= 1297/320; (number of observed mutations for PP in full database/number of observed mutations for PP in restricted database)).

TABLE 4—*Estimated and observed frequencies of fortuitous kinship associations.*

Megaplex	Average SM[a]	Estimated Frequency of FKAs per Megaplex[b]	Estimated No. of FKAs for This Database[c]	Observed No. of FKAs for This Database	Observed Frequency of FKAs per Megaplex
		RESTRICTED DATABASE			
Profiler	5.3	1 in 117	131	162	1 in 94
Profiler Plus	4.7	1 in 346	44	45	1 in 341
Profiler Plus + COfiler	7.3	1 in 1811	8	7	1 in 2194
		FULL DATABASE			
Profiler Plus	4.6	1 in 420	169	177	1 in 403

[a] Derived from Fig. 11 data.
[b] Calculated by dividing Average SM by the number of loci (n) and elevating this value to the nth power.
[c] Obtained by multiplying the estimated frequency per megaplex by the number of pair-wise genotype comparisons carried out with the database.

evidence was sought in data established by other identification modalities.

The DNA identification process was divided into two parts: an automated first step carried out all pair-wise genotype comparisons and identified groups of genotypes that, according to basic Mendelian inheritance rules, met the F1:F2 score requirement; in a second step, the groups of genotypes purported to be related were then manually reviewed and subjected to standard probability and frequency calculations. The automated component part of the identification scheme allowed for the maximal use of the information contained in the genotypic datasets, greatly facilitated the generation of identification leads and allowed for the efficient use of exclusion. This reduced the amount of labor required to carry out genotype comparisons, and performed systematic comparisons unachievable manually. The built-in rare allele and core repeat slip mutation detection routines proved very useful in numerous instances. Frequently, families could be distinguished among the score reports of unidentified victims prior to reception and processing of family relative samples and/or personal effects.

By the end of the identification initiative, 88 victims had been identified by DNA only, 130 had been identified by multiple modalities including DNA. DNA contributed identification evidence for all 218 victims for whom reference samples were submitted. The remaining 11 victims were identified by other means. Interestingly enough, the simple scoring algorithm used in the automated component of the analysis proved to be a very effective screening and scoring tool as all inferred identification leads/confirmation of identification proved to be correct once all available data were compiled.

There were numerous complications during this DNA identification initiative such as lack of personal effects or lack of complete reference donor sets from relatives, which made the use of an automated comparison process even more crucial. FKAs were commonplace in the kinship scoring. A total of six confirmed core repeat slip mutations were encountered. Source attribution of personal effects proved reliable in only 90% of situations.

Despite the large PD values calculated for STR megaplexes, these values describe the probability of randomly encountering any given genotype twice in the population. These values apply to

unrelated individuals, and are therefore of limited utility in the context of kinship analysis. The tabulated megaplex performance statistics for this identification initiative clearly demonstrate that the use of the nine loci comprised in the PR kit would not have been sufficient as a first line megaplex to handle some of the kinship situations encountered. The loci comprised in the PP kit performed much better, but the combined use of PP and CO handled all kinship and parentage situations in this closed population of 539 individuals (229 victims + 310 next-of-kin). Whether 13 loci could prove sufficient to handle all situations in high fragmentation MFIs with a larger number of fatalities would presumably be very much dependent on the specifics of the MFI as it pertains to victim count, the integrity of recovered remains, and reference sample availability.

The Swissair victim identification initiative was the first large-scale MFI to use commercialized STR-based megaplexes. The rapid identification of all victims in just 104 days demonstrated the usefulness of such systems in the processing of large numbers of compromised samples. It also was the first time a bioinformatics tool was constructed to assist with genotype matching and kinship analysis by use of systematic comparative genotyping. This demonstrated the power of such tools in the efficient use of DNA data during identification initiatives that are undertaken in the wake of MFIs. Since the Swissair tragedy, the trend towards systematic use of DNA typing has been sustained through numerous MFIs involving jetliners (Egyptair Flight 990 (Oct. 1999), Alaska Air Flight 261 (Jan. 2000), Air France Flight 4590 (July 2000), American Airlines Flight 587 (Nov. 2001)), the discovery of mass graves in Croatia, and the disaster of the World Trade Center and Pentagon in Sept. 2001, which included four jetliners. In that respect, the kinship analysis software described in this report was upgraded and enhanced to handle the additional complexities of the WTC tragedy, and will be described elsewhere.

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