CHALLENGES POSED WHEN PROCESSING COMPROMISED SAMPLES

Mechthild Prinz, Theresa Caragine, Christopher Kamnick, Dan Cheswick, Robert Shaler
Department of Forensic Biology, The Office of Chief Medical Examiner of the City of New York

Abstract

In the aftermath of the World Trade Center terrorist attack the New York City Office of the Chief Medical Examiner was confronted with approximately 10,000 severely compromised soft tissue samples. At each level of the DNA analysis process, these samples posed various problems. For example, sample collection was a critical step since the contaminated or more degraded outside of a body part had to be avoided. The large sample number required a high throughput extraction method but potential bacterial contamination threatened to saturate any type of DNA capture and release chemistry. Depending on the ratio of bacterial and human DNA an increasingly smaller portion of human DNA would be recovered. The DNA IQ extraction kit (Promega) procedure was adjusted to address this problem. Membrane bound human DNA quantitation assays such QuantiBlot (Applied Biosystems) have been shown to be sensitive to DNA degradation and bacterial contamination resulting in an underestimation of the human DNA content of a sample. Therefore for the compromised tissues the quantitation value was not as reliable in predicting amplification success as it is for fresh samples. The amplification step was also adjusted to optimize sensitivity; for Powerplex 16 (Promega) the maximum recommended cycle number of 10 plus 22 was validated and employed for all samples. The final challenge was the interpretation of partial profiles. Many of the results followed a classic degradation pattern with dropout of the larger loci. In addition the combination of severe degradation and high sensitivity produced stochastic effects and unexpected allelic dropout events even at relatively high peak heights.

Material and Methods

25 mg samples were incubated overnight in 150μL DNA IQ incubation solution, the supernatant was transferred to deep well plates and the DNA was extracted using a Biomek 2000 platform and DNA IQ chemistry according to the Promega protocol (1). Later the extraction was modified to include more Proteinase K and 0.5% SDS in the incubation buffer, the use of triple the amount of magnetic beads and a tip mixing process for the final elution instead of merely shaking the samples. Elution volume was 90 μL. 2μL of each sample were quantitated using QuantiBlot and colorimetric signal development according to the manufacturer's instructions (2). Powerplex 16 amplifications were performed in ABI 9700 thermal cyclers using Promega amplification conditions and the highest recommended cycle number of 10/22 cycles (3,4). Target amount of DNA was 1ng but samples were still amplified using 20μL of extract if the QuantiBlot results were negative. Amplification products were separated on ABI 3100 capillary electrophoresis instruments under standard conditions (5,6). 2μL of PCR product were added to the formamide / ILS6000 (Promega) mix.

Results and Discussion

The initial validation of the DNA IQ procedure for soft tissues showed a very high success rate and high yields of DNA. Partially due to the high DNA content of the samples several checkerboard experiments revealed DNA in the negative controls. All subsequent extractions were therefore performed in checkerboard format to avoid cross contamination. Table 1 shows the comparison of success rates during various stages of the project. The original protocol was modified to deal with the unsatisfactory success rate for the older tissue samples. Soft tissue recovered months after the incident is very likely a substrate for bacterial growth. With an increasing ratio of bacterial DNA to human DNA, the human DNA yield for the DNA IQ extraction procedure will decrease since the magnetic beads only capture a constant amount of DNA.

Based on suggestions made by Promega, the amount of magnetic beads was therefore tripled in order to increase the total DNA recovery. Table 1 illustrates the improvement.

During the initial stages of the World Trade Center work, soft tissue DNA extracts were outsourced for STR typing. All outsourced samples that had originally produced partial profiles, were subsequently amplified with Powerplex 16 in house. Moreover, all previously negative tissues were re-extracted with the DNA IQ modification and also tested with Powerplex 16. While many samples had high DNA yields and complete profiles, most samples showed typical degradation patterns with complete drop out or loss of peak intensity for the high molecular weight loci. Figure 1 depicts such a degradation pattern in a sample with decreasing peak heights and otherwise almost overblown profile. An artifact associated with the DNA locus VWA was regularly observed for profiles with peak heights greater or close to 4000 RFU. The artifact did not only appear for degraded samples, but could also be seen for the positive control DNA. Nevertheless, allele identification was unambiguous.

Since only $1/10^{th}$ of the maximum DNA amplification amount was quantitated, many samples with negative or low quantitation readings could still be successfully typed. Often samples were overamplified, indicating that the QuantiBlot reading actually underestimated the DNA content. This has been previously observed for degraded DNA (7) and DNA containing high amounts of bacterial DNA (8). The quantitation target sequence is only 171 bp long (9) and should not be that susceptible to degradation; another possible factor might be a reduced binding efficiency to the nylon membrane for shorter fragments.

When working with degraded DNA, one should expect allelic drop out (8). There are two possible levels of stochastic effects for degraded samples: the total DNA amount and the presence of intact template. For high molecular weight DNA, allelic drop out occurs only for low levels of DNA with corresponding low peak heights. As presented in Table 2 this does not apply to degraded DNA. 400 allelic drop out events were evaluated by measuring the peak height of the remaining heterozygote allele. The average and median values are in the range observed for sensitivity titrations of high molecular weight DNA (unpublished data), but the maximum peak heights would have been interpreted as true homozygote types. Figure 2 shows an example of an “unexpected” drop out event. At D18S51, the sample appears to be a homozygote since the remaining allele is almost 400 RFU high and a higher molecular weight locus is still intact. The data set in Table 2 does not allow a determination of the frequency of allelic dropout for loci of different lengths. For many profiles, both alleles at the longer loci were absent and thus could not be used for the “allelic” drop out statistics. There seems to be no correlation between the remaining allele peak height and the length of the locus.

The prevalence of allelic dropouts posed a problem for the sample matching and identification process. The software used (MFI-Sys, Genecodes Cooperation) can perform moderate stringency searches and will also flag discrepancies for multiple entries under the same sample ID. For example in August the database contained 3267 samples with multiple sets of results; of these 2938 were consistent with each other and 329 had been flagged for discrepancies. Most of the flagged entries were resolved to be heterozygote/false homozygote scenarios. While the false homozygotes require additional QC reviews the remaining allele might yield useful allele sharing information, especially for partial profiles with only a few loci. In combination with mtDNA a single STR locus can resolve a fortuitous match. In order to be able to automatically upload the data, the following editing rules were formulated to address the allelic dropout issue:

A.) If a profile contains at least 10 reliable looking loci with significant peak heights, any additional loci that show obvious allelic drop out should be removed to avoid unnecessary QC investigations.
B.) If a profile contains less than 10 reliable looking loci with significant peak heights, even loci with obvious allelic drop out and suspicious homozygote loci should be left in to allow matches for low stringency searches. These profiles will require a QC review during the match process.

C.) If a profile doesn't have any heterozygote STR loci, any "stray" homozygote peaks that are below 150 RFU cannot be trusted to represent true alleles and should be removed.
D.) If B.) or C.) would apply but the sample concentration is high enough to allow a reamplification with more DNA, all "homozygote" peaks should be removed and the sample must be reamplified.
E.) Allelic drop out events at the Amelogenin locus should always be edited out in order to avoid a wrong sex determination.

The incentive for these rules was to eliminate obvious false homozgotes while still preserving information for critical samples.

References:

1. Promega Corporation. Tissue and Hair Extraction Kit (for use with DNA IQ) Protocol, Part # TB307. Madison WI, 2002. Promega Corporation.
2. Roche Molecular Systems. QuantiBlot, Human DNA Quantitation Kit. Package Insert. Foster City, 2000, Applied Biosystems.
3. Promega Corporation. Technical Manual, Powerplex 16, Part # TMD012. Madison WI, 2001. Promega Corporation.
4. Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, Tomsey CS, Zachetti JM, Masibay A, Rabbach DR, Amiott EA, Sprecher CJ. Validation of a 16-locus fluorescent multiplex system. J Forens Sci 2002; 47:773-785.
5. Applied Biosystems. ABI PRISM 3100 Genetic Analyzer, User's Manual. Part # 4315834B, Foster City, CA, 2001, Applied Biosystems.
6. Applied Biosystems. ABI PRISM 3100 Genetic Analyzer, Quick start guide for fragment analysis. Part # 4315832C, Foster City, CA, 2001, Applied Biosystems.
7. Alonso A, Andelinovic S, Martin P, Sutlovic D, Erceg I, Huffine E, Fernandez de Simon L, Albarran C, Definis-Gojanovic M, Fernandez-Rodriguez A, Garcia P, Drmic I, Rezic B, Kuret S, Sancho M, Primorac D. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples. Croatian Medical Journal 2001; 42:260-266.
8. Prinz M, Schmitt C. Effect of degradation on PCR based DNA typing. In: Advances in Forensic Haemogenetics. Baer W, Fiori A, Rossi U (eds). Berlin, Heidelberg, New York, 1994; Springer, pp. 375-378.
9. Waye JSW, Willard HF. Structure, organization, and sequence of alpha satellite DNA from human chromosome 17:evidence for evolution by unequal crossing-over and an ancestral pentamer repeat shared with the human X chromosome. Mol Cell Biol 1986; 6:3156-3165.

Tables and Figures

Table 1 Soft tissue typing success rates

Test stage	Number of samples evaluated	Success rate (full or good partial profile)
First month: good quality tissue	N=44	100%
First month: compromised tissue	N=41	63%
4-5 months later	N=80	26%
After DNA IQ re-optimization	N=151	49%

Figure 1

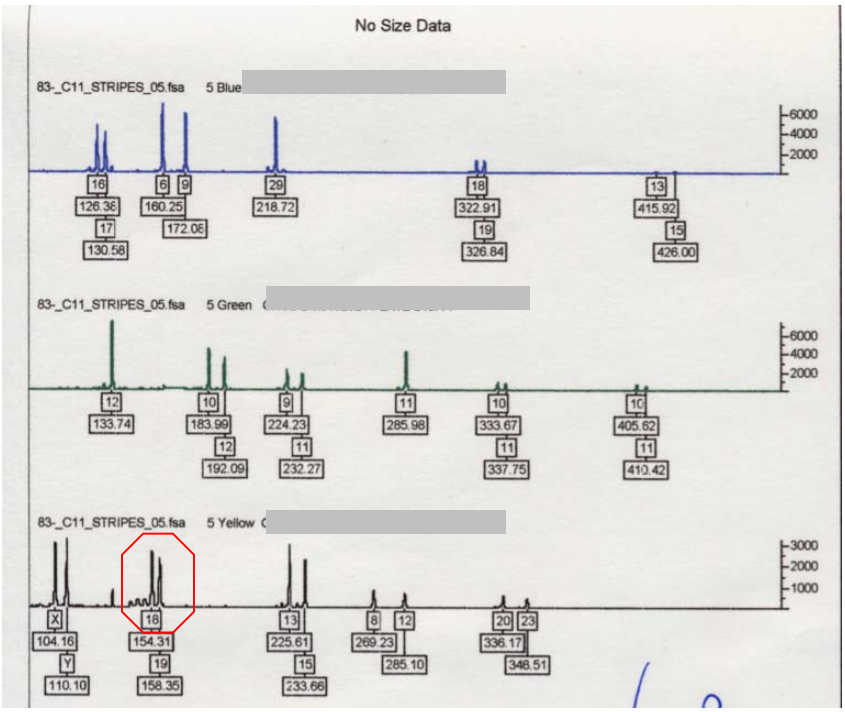

Figure 1 Example for Powerplex 16 electropherogram
While the shorter loci display overblown peak heights in the 4000 – 6000 RFU range, the longer loci show decreased intensities as is to be expected for degraded DNA samples. The octagon marks a VWA artifact that appears to be some type of multiple stutter.

Table 2 Allelic drop out statistic

Loci	n	Average peak height[1]	Median peak height	Maximum peak height
D3S1358	17	133	101	404
THO1	21	113	94	278
D21S11	25	133	128	223
D18S51	25	129	95	382
Penta E	32	124	106	235
D5S818	21	114	108	204
D13S317	29	127	113	344
D7S820	27	123	121	199
D16S539	20	115	95	267
CSF1PO	29	132	114	356
Penta D	25	169	112	612
Amelogenin	22	139	141	260
VWA	23	105	99	228
D8S1179	34	124	104	352
TPOX	22	118	109	201
FGA	28	130	124	231

1: peak height of remaining allele at heterozygote locus in RFU

Figure 2

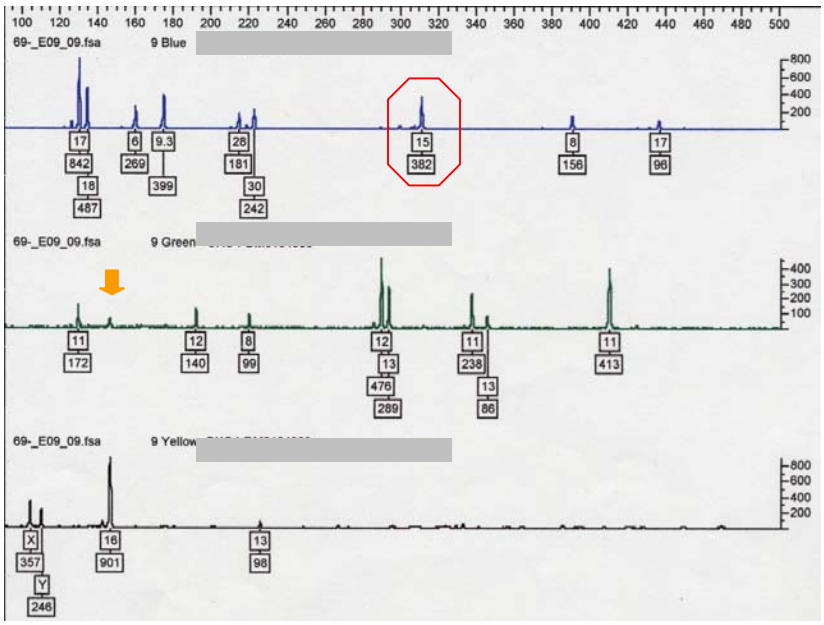

Figure 2 Allelic drop out example
For some loci the second allele peak is still visible (see arrow). An allele 12 should have been detected at locus D18S51 (octagon). The drop out of this allele is difficult to predict since a.) this is the shorter allele, and b.) an even larger locus, Penta E displays a full type.