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Research article

# Challenging DNA: Assessment of a range of genotyping approaches for highly degraded forensic samples

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**Abstract**

It is common in forensic casework to encounter highly degraded DNA samples from a variety of sources. In this category bone and teeth samples are often the principal source of evidential material for criminal investigations or identification of long-deceased individuals. In these circumstances standard STRs are prone to fail due to their long amplicon sizes (since DNA becomes progressively more fragmented as it degrades). To successfully resolve such cases alternative markers can be used and until recently the only other tool available was mitochondrial DNA, which despite being more resistant to degradation, is much less informative. A rapidly developing approach to analyzing degraded DNA is the typing of loci from short-amplicon PCR products based on markers such as mini-STRs and autosomal SNPs. We have performed an analysis of several cases with naturally degraded DNA using established STRs plus mini-STRs and autosomal SNPs in order to make an objective comparison of the performance of each method using challenging DNA. The main aim was to establish the benefits and drawbacks of each marker set to help the practitioner choose the DNA analysis method most suited to the circumstances of each case.


*Keywords:* STRs; Degraded DNA; Short-amplicon STR analysis; SNP typing

## 1. Introduction

It is common to encounter highly degraded DNA samples in a criminal investigation or the identification of long-deceased individuals. Most forensic laboratories have experienced situations where the DNA is so degraded that normal PCR amplification gives inconclusive results. The quality of genotyping depends largely on the degradation processes the sample has been exposed to and these affect typing success in different ways: agents can affect the DNA structure itself through the action of nucleases or oxidative damage, while indirectly, the inhibitory effects of co-extracted agents such as humic acid or degradation by-products can hinder the PCR reaction. The extent of the degradation process depends on two factors: time and environmental conditions [1]. Degradative processes accumulate with time while environmental conditions (temperature, humidity, pH, soil chemistry) modify the rate and aggressiveness of degradation. Both factors interact in a complex way so there is no direct correlation between time since death/deposition of material and the extent of degradation, making it very difficult to set specific rules for the treatment of any one sample. Fortunately several short-amplicon genotyping approaches have been recently developed specifically for the analysis of degraded DNA. This study compares the performance of established and novel genotyping methods in a range of challenging cases with naturally degraded DNA.

## 2. Materials and methods

We analyzed 15 separate skeletal samples from submitted case-work, in a range of conditions, each assessed from the performance of standard STR typing. All cases originated from NW region of Spain: characterized by high annual rainfall, mild temperatures, and organic-rich, acidic soil. Surviving relatives were analyzed to obtain reference profiles when available. Prior to DNA extraction, bones or teeth were thoroughly cleaned with a scalpel/sandpaper. Except for the most degraded samples, extraction was based on the phenol–chloroform method with *Centricon*® column purification. For the most degraded

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material we used a second method based on the ancient DNA protocol of Lalueza-Fox et al. [2] modified to enhance DNA yield. All extracts were quantified with the real-time PCR *Quantifiler*® human DNA kit (Applied Biosystems: AB). An internal PCR control (IPC) in each *Quantifiler*® reaction identifies material containing PCR inhibitors. Standard STR typing comprised *AmpFlSTR Identifiler*® (AB) and *Power-Plex16* (Promega) with extract dilutions: neat, 1 in 2, 1/4, 1/8 and 1/16 in tandem PCRs. Two mini-STR sets were: *MiniNC01*, developed at NIST [3], and *AmpFlSTR MiniFiler*® (AB). SNP typing consisted of two assays: the SNP*for*ID 52plex human identification SNP set [4] and the SNP*for*ID 34plex population informative AIM-SNP set [5]. Both sets use an initial PCR followed by a multiplexed primer extension (52plex comprises tandem 23 and 29plex extension reactions). SNP assays used undiluted DNA samples throughout.

## 3. Results

Table 1 outlines quantification data and % genotyping success from each sample. The most significant findings came from a comparison of genotyping success with data from the *Quantifiler*® IPC. IPC values higher than 28 indicate the presence of inhibitors which themselves can affect the quantification, so values obtained in these circumstances are less indicative of the actual DNA levels in an extract. Furthermore, values suggesting high DNA concentrations do not often equate to sufficient quantities of intact high molecular weight target to ensure successful PCR of standard STRs. We countered inhibitory effects by diluting extracts so although less DNA was applied, inhibition was reduced. Most samples gave complete profiles with each multiplex once inhibition–reducing methods were applied. Table 1 shows that simple dilution of the extract is, in nearly all cases, enough to enhance the genotyping success rate suggesting that inhibitors play a critical role in reducing PCR efficiency in severely degraded samples. However long amplicon systems (leftmost in Table 1) appear to be more affected by PCR inhibitors, either due to a lower initial undegraded target concentration, a less efficient DNA polymerization, or both. In contrast SNP typing shows relative immunity from PCR inhibition effects since these systems are likely to benefit from a higher initial concentration of intact target plus more efficient (shorter) polymerization steps.

If amplicon length is an important factor with or without inhibition control then the success rate should be expected to rise as amplicon size diminishes. This was observed with the standard amplicon STRs, since *Powerplex16* showed the highest overall failure rate. This trend continued as the degree of degradation rose, and longer *Identifiler*® loci failed to amplify (amplicons up to 380 bp) followed by *MiniFiler*® (amplicon up to 300 bp). The performance of mini-STR loci between 100 and 300 bp does not differ markedly, however STR products below 100 bp, notably those of *MiniNC01* (all amplicons <120 bp) appear to be resistant to the most aggressive degradation in the samples of this study. It can also be suggested that a small-scale triplex PCR using amplicons much shorter than average is likely to be more efficient when intact target DNA is at low levels in the extract. In comparison SNP multiplexes clearly do not need to be small-scale in scope to achieve efficient amplification of highly degraded DNA. An additional advantage of the SNP assays developed by SNP*for*ID is that the genotyping reaction is separated from the initial PCR so amplicons of similar length, often much shorter than 100 bp, can be combined with ease.

The above findings suggest that for most degraded material standard STR typing methods will suffice if inhibition is properly assessed and controlled prior to PCR. However a further level of degradation, characterized by extremely aggressive environmental conditions over long periods of time can present the most challenging analyses. This applies to two femurs we analyzed: *78p03* was from a 35-year internment in a tomb with warm, damp, acid conditions, both epiphyses were missing and the bone had a loose, sawdust form with extensive

Table 1
Genotyping success of 15 cases involving challenging DNA, with extracts in order of increasing success rate using Powerplex16 (this assay comprises longest overall amplicon sizes)

| Sample | IPC value | Quantity DNA (ng/μl) | Powerplex16 | | Identifiler® | | MiniFiler® | | 52plex auto1 (23plex PCR) | | 34plex AIM-SNP set | | 52plex auto2 (29plex PCR) | | MiniNC01 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Inhibition | Corrected | Inhibition | Corrected | Inhibition | Corrected | Inhibition | Corrected | Inhibition | Corrected | Inhibition | Corrected | Inhibition | Corrected |
| 70-06 | ND | 0.04 | 0 | 0 | 0 | 0 | 30 | 50 | 85 | 100 | 90 | 100 | 93.7 | 100 | 100 | 100 |
| 78P03 | ND | 0.53 | 0 | 0 | 9.3 | 9.3 | 38.8 | 38.8 | 95.6 | 100 | 100 | 100 | 96.5 | 100 | 100 | 100 |
| 71P04 | 28.03 | 0.67 | 62.5 | 62.5 | 78.12 | 87.5 | 100 | 100 | 82.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 126P04 | 28.43 | 1.03 | 78.1 | 78.12 | 62.5 | 100 | 100 | 100 | 84.8 | 100 | 95.6 | 95.6 | 100 | 100 | 100 | 100 |
| 122P04 | ND | 5.88 | 87.5 | 87.5 | 0 | 100 | 100 | 100 | 97.8 | 100 | 100 | 100 | 93.1 | 100 | 100 | 100 |
| 23P04 | 27.73 | 0.03 | 87.5 | 93.75 | 31.25 | 100 | 100 | 100 | 95.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 72P03 | 31.69 | 0.6 | 93.75 | 93.75 | 81.25 | 84.37 | 44.4 | 44.4 | 95.6 | 100 | 100 | 100 | 93.1 | 100 | 100 | 100 |
| 77P04 | 28.56 | 0.9 | 87.5 | 96.87 | 93.75 | 100 | 100 | 100 | 67.4 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 50p04 | 27.76 | 0.12 | 87.5 | 100 | 43.75 | 100 | 88.9 | 88.9 | 100 | 100 | 97 | 97 | 100 | 100 | 100 | 100 |
| 12P05 | 28 | 0.16 | 100 | 100 | 87.5 | 100 | 100 | 100 | 82.6 | 100 | 100 | 100 | 96.6 | 100 | 100 | 100 |
| 45P04 | 28.12 | 0.72 | 96.9 | 100 | 100 | 100 | 88.9 | 88.9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 24P05 | 28.3 | 0.98 | 100 | 100 | 87.5 | 100 | 100 | 100 | 86.9 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 11P05 | 35.55 | 2.93 | 93.75 | 100 | 56.25 | 100 | 100 | 100 | 71.7 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 105-05 | 35.15 | 8.63 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 5P04 | 35.49 | 19.51 | 93.75 | 100 | 25 | 100 | 100 | 100 | 73.8 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Values for each multiplex denote % success as proportion of full genotypes observed in undiluted extract (inhibition) and at optimum dilution (corrected). Multiplexes arranged in ascending order left to right of total genotyping success. ND = not determined, IPC = internal PCR control.

mould growth; *70–06* was from skeletal remains half buried for 10 years in forest soil (damp, acid and organic-rich conditions) that were discovered after a severe forest fire which badly charred the remaining tissue/bone surfaces. High molecular weight DNA was absent from samples and all standard STRs failed, together with most mini-STR loci. In contrast, successful amplification of the shortest amplicon mini-STRs and all SNP multiplexes indicated that short, fragmented DNA was present in enough quantity for efficient PCR of these systems. Run in parallel for these samples; the standard protocol DNA extracts amplified poorly compared to those from the ancient DNA protocol (respectively: 22.2% vs. 44% success with *MiniFiler*® and 85% vs. 100% with SNP typing). The ancient DNA extraction method had a major impact on DNA recovery and typing success for *MiniNC01* and autosomal SNPs. Improved DNA yield with the ancient DNA protocol also helped to control inhibition in all systems by allowing greater levels of dilution.

## 4. Conclusions

An optimum analysis method for degraded DNA can be chosen between standard STRs and short-amplicon STRs combined with SNP typing depending on several key factors: the likely state of degradation, initial visual exploration of the sample upon receipt, volume of extract obtained and quantification results, particularly IPC values detected. Generally DNA fragments up to 300 bp should be present (even in low concentration), and these can be successfully amplified with any approach if inhibition is properly managed. Our analysis of two cases with extremely degraded material indicates that SNPs and small-scale mini-STR assays amplifying DNA from extraction procedures optimized for both yield and inhibition, offer the best approach.

## Conflict of interest

None.

## References

[1] J. Burger, S. Hummel, B. Herrmann, W. Henke, Electrophoresis 20 (1999) 1722–1728.
[2] C. Lalueza-Fox, J. Bertranpetit, J.A. Alcover, N. Shailer, E. Hagelberg, J. Exp. Zool. 288 (2000) 56–62.
[3] D. Michael, M. Coble, J.M. Butler, J. Forensic Sci. 50 (2005) 43–53.
[4] J.J. Sanchez, C. Phillips, C. Børsting, K. Balogh, M. Bogus, M. Fondevila, C.D. Harrison, E. Musgrave-Brown, A. Salas, D. Syndercombe-Court, P.M. Schneider, A. Carracedo, N. Morling, Electrophoresis 27 (2006) 1713–1724.
[5] C. Phillips, A. Salas, J.J. Sanchez, M. Fondevila, A. Gómez-Tato, J. Álvarez-Dios, M. Calaz, M. Casares de Cal, D. Ballard, M.V. Lareu, Á. Carracedo, The SNP*for*ID Consortium, Forensic Sci. Int. Genetics 1 (2007) 273–280.