Optimisation of miniSTR analyses allows the study of ancient degraded samples

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The vertiginous progress in molecular biological techniques has promoted the retrieval and analysis of DNA from very diverse ancient specimens. This has broadened the scope of possibilities for Genetic Anthropology enabling the study of a wide range of samples of historical interest, permitting the identification of important historical persons and the establishment of familial relationships as well as population migration studies. The development of new methodologies and the adoption of suitable laboratory practices and approaches makes it possible to obtain results, of accreditation authenticity, from a number of challenging samples that until recently were not amenable to analysis. However, along with the technical progresses, this new field has revealed certain peculiarities and drawbacks.

The special characteristics of ancient DNA (aDNA) determine the approaches chosen to study it. After overcoming inhibition, if present and possible, the next challenge is the great degree of molecular fragmentation. In this respect, mitochondrial DNA (mtDNA) was readily chosen as the preferred target since its elevated copy number in the cells makes it more likely to survive than nuclear DNA. Thus, even in cases where little or no nuclear DNA was present, mtDNA was deemed to be the panacea that permitted the obtaining of genetic information from difficult degraded samples.

We have analysed a set of medieval human remains (carbon dated to 960-1300 AD) where a wide range of conditions were found, in terms of quantity of nuclear DNA and presence of PCR inhibitors and contamination. Much effort has been made in obtaining clean, unambiguous mtDNA sequences. In a few cases, the attempts have been successful, although the authenticity of the sequences obtained cannot be guaranteed as molecular damage of DNA occurs inexorably over time and some base changes could have occurred. In other cases, in spite of following the strictest procedures to eliminate sample contamination and to avoid further contamination in the laboratory, contamination in the DNA extracts or in the amplified products could not be fully avoided and endogenous mtDNA sequences of the sample were completely masked and impossible to discern.

To date many research groups have reported successful results on mtDNA from ancient samples from very diverse origins. One of the most outstanding cases is the achievement of the mitochondrial genome of the Neanderthal10, and some others from extremely well preserved samples such as those kept in permafrost12. There is no doubt about the advantages of the study of mtDNA. Nevertheless, it is important to put in perspective its applications in the most general sense. Firstly, when studying animal species different from human, it is generally possible to detect exogenous contamination from human sources, which is not always the case with human samples. Secondly, the excellent degree of preservation conferred by constant low [below zero] temperatures is not the most common situation in the majority of cases of study. Thirdly, many of these exciting reports are the result of laboratory consortiums and the common effort of, sometimes, up to fifty researchers working with cutting-edge, and therefore, highly expensive, technology and equipments. This is not the reality of the great bulk of laboratories working in
forensic genetics nowadays, as in many cases they could not undertake such investments. Moreover, in many cases of study on human ancient DNA the ultimate goal is to establish familial relationships, which may be limited if only an uniparental genetic marker such as mtDNA is used.

Alternatives to mitochondrial DNA analysis exist. The analysis of short tandem repeats (STRs), which have the greatest discriminating power, is usually unsuccessful when dealing with degraded and scarce samples. The quest for extracting information from nuclear DNA, even in the most adverse situations, has led to the development of markers of reduced size, such as the miniSTRs, or even to the analysis of single nucleotide polymorphisms (SNPs). MiniSTRs are considered to be the most effective approach for degraded DNA. Commercial laboratories have readily developed kits containing informative miniSTRs that enable the study of difficult samples while allowing comparisons with the standardized core loci STRs. Moreover, the recently introduced generation of kits not only have reduced the size of amplicons but also improved buffer formulations and primer synthesis processes, resulting in an increase in the sensitivity and success rates in the presence of inhibitors, high quality profiles and low baselines.

In our experience of genotyping medieval human remains, we have studied autosomal STR loci using a standard human identification STR kit (AmpFISTR® Identifier PCR Amplification Kit, Applied Biosystems®) in cases where the amount of nuclear DNA retrieved allowed its use. However, in many of them, even when the amount of DNA was adequate for conventional STR analysis, DNA was degraded to the point that the Identifier® kit would fail. MiniSTR loci were then typed using the AmpFISTR® MiniFiler™ PCR Amplification Kit (Applied Biosystems®). MiniSTRs have proven to be the best approach in most cases, obtaining full or partial miniSTRs profiles when conventional STRs failed. The advent of the AmpFISTR® NGM™ PCR Amplification Kit (Applied Biosystems®) further maximizes the chances of obtaining a result from severely degraded samples, even in the presence of high levels of inhibitors.

Forensic scientists have used different strategies to increase the sensitivity of routine typing systems available in order to augment the molecular information obtained from degraded skeletal remains, which have been grouped under the term “low copy number” (LCN) typing. The MiniFiler™ kit is also amenable of optimization when the recommended working conditions are not successful for typing especially valuable samples. An interesting case in our lab was a medieval bone sample that yielded DNA amount amenable of being analysed by the MiniFiler™ kit, but no results were generated using either the standard conditions or the same method with increased number of cycles (36 instead of 30; Figure 1a). Nevertheless, seven miniSTRs (out of 8) plus the amelogenin marker were obtained after further optimization of the typing protocol which consisted in doubling the amount of PCR master mix and primer mix and adding extra magnesium (0.5 µL 50 mM MgSO4) and Taq polymerase (0.5 µL AmpliTaq® Gold DNA Polymerase, Applied Biosystems) to the PCR reaction in a final volume of 38 µL (Figure 1b). Cycling conditions were performed following the manufacturer’s recommendations, but six additional PCR cycles were used (36 cycles instead of 30). The DNA amount per PCR reaction was within the recommended range [0.50–0.75 ng]. Another example is presented in Figure 2.
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Raising the number of cycles greatly increases PCR sensitivity. Nevertheless, when the input DNA is too low, there is a risk of fluctuation effects due to stochastic sampling of the two alleles present in a heterozygous individual, generally manifested as a peak imbalance of the alleles. For this reason, cautious interpretation of LCN STRs results is needed. On the other hand, it is generally possible to produce results in duplicates or triplicates, and ideally, more than one sample from the same individual can be analysed, thus increasing the confidence and robustness of the analysis.

The use of highly optimized miniSTR typing systems allows the genotyping of ancient human remains and proves to be a valuable tool for Genetic Anthropology, and in many cases becomes an excellent alternative to mtDNA analysis as a first-choice approach in genotyping ancient degraded samples. The possibility to obtain information from nuclear DNA from compromised samples is very well received since the STR loci are the most informative genetic markers for identity testing.

Figure 1. A medieval bone sample was extracted with a silica DNA binding method. STR amplification was performed with the MiniFiler™ kit. (a) Amplification with the MiniFiler™ kit under the recommended conditions produced no results. (b) Seven out of eight miniSTRs plus the amelogenin marker were obtained after optimization of amplification with the MiniFiler™ kit (see text).
Figure 2. Example of miniSTR analysis on a case study. A medieval bone sample was extracted with a silica DNA binding method. STR amplification was performed with both Identifiler® and MiniFiler™ kits. [a] Amplification with the Identifiler® kit produced no results. [b] Amplification with the MiniFiler™ kit under the recommended conditions produced a partial profile. [c] Seven out of eight miniSTRs plus the amelogenin marker were obtained after optimization of amplification with the MiniFiler™ kit (the number of cycles was raised from 30 to 35 and 0.8µL Taq polymerase were added per reaction).
References


