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The top 10 list: criteria of authenticity for DNA from ancient and forensic samples

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1. Introduction

Preserved archeological and paleontological specimens contain genetic information that has the power to elucidate the recent evolutionary history of humans, domesticates, and the pathogens they harbored. In addition, DNA from forensic samples is important in sample identification and crime solving. However, the DNA of both fossil and forensic remains can be heavily degraded through hydrolytic cleavage and oxidative base damage, limiting its successful retrieval and amplification. Obtaining authentic DNA sequences from both ancient and forensic remains presents extreme technical difficulties due to the minute amounts, and degraded nature, of DNA along with the exceptional risk of contamination. I reiterate here [1] a rigorous set of 10 criteria to ensure, to the greatest extent possible, meticulous replication and authentication of degraded DNA templates ubiquitous in all ancient and forensic samples.

2. DNA degradation in postmortem matrices

Postmortem probably the single most important factor in the degradation of DNA is the rate at which cellular nucleases degrade the endogenous DNA. The DNA must then face the bacterial, fungal and insect onslaught, which can be effective but is often incomplete [2]. Remaining DNA is still then the subject of chemical degradation via hydrolysis and oxidation [7].

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2.1. Hydrolytic DNA damage

The DNA molecule is particularly prone to hydrolytic damage [7,13]. Althoughthe phosphodiester bonds are relatively stable, the glycosidic base sugar bond is the main target of direct hydrolytic attack, leading to base loss, usually through depurination, and shortly thereafter, a single stranded nick at the abasic site. DNA bases with secondary amino groups such as adenine, cytosine, 5-methylcytosine and guanine undergo deamination, the hydrolytic loss of their amino groups, resulting in hypoxanthine, uracil, thymine, and xanthine, respectively. Cytosine residues are particularly prone to this reaction and result in uracil leading to C–G to T–A base pair transversions [3].

Ancient DNA is hydrolytically damaged. Most of the DNA extracted and amplified from fossil as well as sub-fossil remains is between 100 and 500 bp in length [4,10]. This is likely to be due to depurination and hydrolytic cleavage of the phosphate ester bonds and direct strand cleavage due to drying. In addition, it has been shown recently that cytosines of DNA extracted from fossil cave bear teeth have been extensively deaminated, confounding the interpretation of the correct endogenous sequence [4].

2.2. Oxidative DNA damage

While the double strand does afford some protection of the bases to free radical attack, the 3–4′ carbon bond of the deoxyribose is susceptible to oxidation leading to ring fragmentation and strand scission. The major site of oxidative attack on the DNA bases are the 5–6 C=C double bonds of both pyrimidines, and the imidazole ring in purines, both leading to ring fragmentation [3,7]. A majority of these oxidative base products are replication blocks and are thus not by-passed with standard Taq polymerases used in PCR [15].

Ancient DNA is oxidatively damaged. It has been previously shown that DNA extracted from fossil remains is susceptible to cleavage with endonuclease IV and endonuclease III, and thus contains oxidative labile sites [10]. More recently it was shown with gas chromatography/mass spectrometry (GC/MS) that many paleontological specimens from a diverse range of environments and ages contain oxidized base moities. Interestingly, samples with higher amounts of two oxidized pyrimidines, namely, 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd) and 5-hydroxyhydantoin (5-OH-Hyd), were not amplifiable via the polymerase chain reaction [5].

Thus, DNA extracted from fossil and even sub-fossil remains contains damage similar to that accrued in normal cellular DNA in-vivo. In addition, there is no correlation between DNA recovery and the age of most specimens but rather with the temperature of the site. Samples from cold and dry climates tend to preserve macromolecules for longer periods of time due to slower rates of reactions. Thus, while forensic samples are much younger than ancient samples, these will presumably contain similar types of damages.

To ensure authentic DNA results from both ancient as well as forensic samples, I have listed below 10 ‘‘criteria of authenticity’’ [1]. To ensure reproducibility within and between ancient and forensic labs, it would be beneficial if all labs attempted to follow these guidelines as much as possible.

2.3. Criteria of authenticity "The Top 10 list"

2.3.1. Physically isolated work area

To avoid contamination, it is essential that all DNA work be carried out in an isolated lab dedicated to low copy number samples. A separate building, if possible where no genetic work is being carried out, would be the best possible situation. Post-DNA extraction, all PCR reactions can be set up in this laboratory space and then moved to a normal lab for subsequent typing and sequencing.

2.3.2. PCR control amplifications

It is wise to conduct environmental PCRs, every so often, that is the set up of 20 non-template PCRs containing aliquoted reagents along with water. This way the lab has a record of the level of contamination at several points in time. Naturally, multiple DNA extraction and PCR controls should also be performed alongside actual template samples to detect sporadic or low-copy number contamination, although carrier effects do limit their efficacy. All contaminated results should be reported, and positive controls should generally be avoided as they provide a contamination risk.

2.3.3. Test the molecular behaviour

Most of the DNA in ancient and forensic samples is heavily cleaved through hydrolysis and oxidation as well as cross-linked to proteins. A consequence of this is the restricted amplification of PCR products generally larger than 1000 bp. Thus, PCR amplification strength should be inversely related to product size so long as the primers used are roughly equal in sensitivity.

2.3.4. Quantitation

The copy number of the DNA target should be assessed using real time PCR [8] or through the design of a competitive PCR assay [6,9]. When the number of starting templates is low (<1000), it may be impossible to exclude the possibility of sporadic contamination, especially for human DNA studies. Levels of human contamination can range drastically in samples as well as in standard reagents used for DNA extraction as well as PCR.

2.3.5. Reproducibility

Results should be repeatable from the same, and different, DNA extracts of a specimen.

2.3.6. Clone

Direct sequencing of PCR products should be verified by cloning amplified products and sequencing ten clones to determine the ratio of endogenous to exogenous sequences, and damaged induced errors.

2.3.7. Independent replication

The ultimate replication involves the extraction, amplification and sequencing of separate samples of the same specimen in independent laboratories. These experiments

are particularly important with human remains or when novel and unexpected results appear.

2.3.8. Biochemical preservation

Indirect evidence for DNA survival in a specimen can be provided by assessing the total amount, composition, and relative extent of diagenetic change in amino acids and other residues [11,12]. In the case of forensic samples, where protein residues may still be in excellent shape it may be wise to look at another marker such as the extent of fatty acid oxidation or lipid peroxidation.

2.3.9. Associated remains

In studies of human remains where contamination is especially problematic, evidence that DNA targets survive in associated faunal material can provide critical supporting evidence. Faunal remains also make good negative controls for human PCR amplifications.

2.3.10. Phylogenetic sense

Reproducible sequences should in the end be placed in a tree phylogenetically with other known haplotypes in order to ensure that as the final criteria they are indeed authentic. This approach is particularly important in the case of detecting mitochondrial pseudogenes in the nucleus (numts) [14].

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