Continuing concerns about the rigor of research on ancient DNA and that "high-profile journals continue to publish studies that do not meet the necessary controls" prompt a list summarizing "criteria of authenticity" required for work published in this area. The role of the polio vaccination program carried out in Central Africa in the late 1950s in the origin of HIV and AIDS (as posited in the book *The River*) is hotly debated. And "the myth...that efficient use of nuclear resources is a proliferation threat" is challenged, and it is suggested that "electricity produced from existing nuclear by-products would be equivalent to that needed by the United States, at present use rates, for hundreds of years."

Ancient DNA: Do It Right or Not at All

At the recent 5th International Ancient DNA Conference in Manchester, U.K., reported by Erik Stokstad in his News Focus article "Divining diet and disease from DNA" (28 Jul., p. 530), one presentation boldly opened with the claim that the field was now mature and could move ahead with confidence. This optimism is unfounded, as demonstrated by the notable absence of "criteria of authenticity" from many presentations at the conference. Ancient DNA research presents extreme technical difficulties because of the minute amounts and degraded nature of surviving DNA and the exceptional risk of contamination. The need to authenticate results became obvious in the mid-1990s when a series of high-profile studies were shown to be unrepeatable (*1*). For example, DNA reputed to come from a dinosaur (*2*) was actually contamination by a human mitochondrial gene insertion in the nucleus (numt) (*3*). Over the ensuing years, criteria have been developed and put into practice by some practitioners in the field. Regrettably, despite the recommendation that such criteria be routinely applied (*4–6*), high-profile journals continue to publish studies that do not meet the necessary controls (*7*), and many new researchers fail to utilize them. To publicize these standards, we summarize the key criteria below.

Physically isolated work area. To avoid contamination, it is essential that, prior to the amplification stage, all ancient DNA research is carried out in a dedicated, isolated environment. A building in which large amounts of the target DNA are routinely amplified is obviously undesirable (*8*).

Control amplifications. Multiple extraction and PCR controls must be performed to detect sporadic or low-copy number contamination, although carrier effects do limit their efficacy (*4, 9*). All contaminated results should be reported, and positive controls should generally be avoided, as they provide a contamination risk.

Appropriate molecular behavior. PCR amplification strength should be inversely related to product size (large 500– to 1000–base pair products are unusual). Reproducible mitochondrial DNA (mtDNA) results should be obtainable if single-copy nuclear or pathogen DNA is detected. Deviations from these expectations should be justified; e.g., with biochemical data. Sequences should make phylogenetic sense.

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Human paleofeces, 8000 to 500 years old, from Hinds Cave, Texas, USA, is a good source of DNA for both humans and the food they ate.

Reproducibility. Results should be repeatable from the same, and different, DNA extracts of a specimen. Different, overlapping primer pairs should be used to increase the chance of detecting numts (*10*) or contamination by a PCR product.

Cloning. Direct PCR sequences must be verified by cloning amplified products to determine the ratio of endogenous to exogenous sequences, damage-induced errors, and to detect the presence of numts. Overlapping fragments are desirable to confirm that sequence variation is authentic and not the product of errors introduced when PCR amplification starts from a small number of damaged templates (*11*).

Independent replication. Intra-laboratory contamination can only be discounted when separate samples of a specimen are extracted and sequenced in independent laboratories. This is particularly important with human remains or novel, unexpected results.

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 (*12, 13*).

Quantitation.* The copy number of the DNA target should be assessed using competitive PCR (*4, 11*). When the number of starting templates is low (<1,000), it may be impossible to exclude the possibility of sporadic contamination, especially for human DNA studies.

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

We recognize that adherence to these criteria as part of routine good practice is both expensive and time-consuming. However, failure to do so can only lead to an increasing number of dubious claims, which will bring the entire field into further disrepute. If ancient DNA research is to progress and fulfill its potential as a fully-fledged area of evolutionary research, then it is essential that journal editors, reviewers, granting agencies, and researchers alike subscribe to criteria such as these for all ancient DNA research.

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*For important discoveries, additional criteria are also essential.

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