

because the extraction methods used in this analysis were inadequate, or because we were unable to overcome PCR inhibitors once the DNA was extracted. However, we used a variety of methods to overcome PCR inhibition, including an additional Proteinase K clean-up step to further break down proteins in the samples, dilutions of the extractions reduce the proportion of inhibitors relative to DNA, and the use of BSA in the PCR reaction (BSA may bind to inhibitors, thus inhibiting the inhibitors).

Moreover, any DNA amplification we obtained seems to have been a product of exogenous human contamination. This is immediately clear in two cases: (1) when the PCR negative controls are contaminated, and (2) when the PCR product is sequenced and matched to an investigator (e.g., an excavator or other analyst). The first case implies that at the very least the reagents used in the PCR are contaminated, and reagents become easily contaminated through multiple uses. In this case, we threw out the old PCR reagents and performed a new PCR with fresh reagents. The second case demonstrates that even under the most stringent conditions, modern contaminating DNA is problematic in ancient DNA analysis.

Finally, it is possible that an amplified DNA product is the result of exogenous human contamination despite the fact that negative controls yield no amplification. If very low levels of exogenous DNA exist in the PCR reagents, and there are more "samples" than PCR negative controls, chances are the contamination from the PCR reagents will show up in the samples, rather than in the PCR controls. For similar sampling reasons, low levels of exogenous DNA in DNA extracts and disposable pipette tips, tubes, etc., also will amplify sporadically, from one PCR to another. Thus, in addition to the use of controls, we monitor the results of positive amplifications by sequencing the amplification products. For example, PCRs 14, 15, 16, and 17 showed no positive amplification of PCR or extract controls, yet the sequences (shown in [Appendix 3](#)) were an exact match of our laboratory technician.

To conclude, we attempted a variety of techniques to both extract DNA from the sample and reduce the effects of potential PCR inhibitors. When DNA amplified, it was always from an exogenous source. This, combined with the fact that even very low levels of contaminating DNA will preferentially amplify when the sample itself contains little or no DNA of its own, leads us to conclude that little or no DNA remained in the Kennewick samples transferred to our laboratory. However, our conclusion should not preclude further DNA testing using future novel methods on other, perhaps more DNA-rich, bone samples from the Kennewick remains. We are still of the belief that teeth are the best source for clean DNA free of exogenous contamination and would still argue for testing of the teeth (even with the caveat that X-rays can damage DNA and all but two of the teeth were apparently X-rayed).

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