

LETTER TO THE EDITOR

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Response from the authors to the letter from Ferri et al.

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Dear editor, we previously described that genomic sequences could be amplified from mouse, but not human, hair shafts by microwave irradiation followed by PCR [1]. Ferri et al. [see this issue] have suggested that the positive amplification that we observed was due to contamination with cells from mouse saliva or urine, rather than amplification of DNA from the mouse hair shafts themselves.

Ferri et al. drew their conclusions from 2 points of their experience:

1. After mouse shafts were pre-processed with a decontamination protocol by Jehaes et al. [2], both microwave-irradiated samples and lysate samples were negative for PCR amplification.

The decontamination protocol [2] was as follows: the hairs were incubated at 56 °C for 2 h in 100 mM NaCl, 10 mM EDTA, 0.4% SDS and 250 µg/ml of proteinase K and washed twice, once in 0.9% NaCl and once in 100% ethanol.

2. As for human hair shafts, microwave-irradiated samples were negative for PCR (this is the same result as ours). When human hair shafts were artificially contaminated with different individual's saliva, microwave-irradiated samples were positive for PCR of the saliva's genotype. After decontamination, lysate samples were only positive for PCR of hair donor mtDNA genotype.

We wish to make note of 2 important points: 1) Ferri et al. show no direct evidence that mouse hair shafts were, in fact, contaminated with cells or cell debris from saliva or

urine. 2) Furthermore, they do not show that mouse hair shafts are resistant to the lysis conditions used in the decontamination protocol.

Their claim of contamination of mouse hair shafts was based on analogy to the observation for human hair shafts. Therefore, contamination can only be a matter of speculation in mice until further experiments are carried out. Even if contamination occurs in mouse hair shafts, it is doubtful that the amplification products from irradiated mouse hair shafts were only derived from contaminant cells. Ferri et al. considered that everything removed by the decontamination protocol was a contaminant. As shown above, the decontamination procedure evaluated with human hair shafts, is a standard lysis condition for extracting tissue DNA. The authors (Jehaes et al.) actually noticed that hair root nuclear DNA (nuDNA) was lost by this method and that only mitochondrial DNA (mtDNA) of hair shafts was amplified [2]. The authors did not clearly state that the nuDNA of hair shafts was resistant to this method. Considering biochemical and biological differences between mouse and human hair shafts, it remains possible that mouse hair shaft DNA is lost by this decontamination process.

Thus, we consider it still a matter of conjecture and an over-interpretation of the currently available data to assume that microwave PCR of mouse hair shafts amplifies only contaminant DNA.

References

1. Ohhara M, Kurosu Y, Esumi M (1994) Direct PCR of whole blood and hair shafts by microwave treatment. *Biotechniques* 17:726–728
2. Jehaes E, Gilissen A, Cassiman JJ, Decorte R (1998) Evaluation of a decontamination protocol for hair shafts before mtDNA sequencing. *Forensic Sci Int* 94:65–71

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