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LETTER TO THE EDITOR

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Correct mitochondrial L-strand sequencing after C-stretches

W. Parson et al.
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Dear Sir,

We have read the paper by Parson et al. [5] with great interest. Our experience with sequencing the mitochondrial d-loop in 141 individuals from a German population seems to match the information of the authors. We have also noticed that forward sequencing matching the mtDNA light strand usually produces better results than reverse sequencing and that T to C transitions in the positions of nt16189 and nt00310 occur quite frequently, i.e. 12.8% and 0.6%, respectively. The formation of C-stretches that consist of more than 10 cytosines usually results in a length heteroplasmy within the tract [2]. This heteroplasmy produces a characteristic blurred sequence in nucleotides beyond C-stretches. Until recently made the basecalling of the sequence following a C-stretch exclusively by sequencing the H-strand which we find to have shortcomings. We have recently established a database in Magdeburg where data for the comparison of the frequencies of d-loop sequences are compiled for forensic purposes (http://www.d-loop-base.de).

A precondition has been determined by the participating institutes that only those sequences that have been confirmed by L- and H-strand sequencing should be accepted. However, the failure of the recording process after poly-C-stretches led to the circumstance that these problematic sequences were only recorded as H-strand sequencing. The occurrence of blurred signals as a result of poly-C-stretches leads to major sequencing problems in all population studies. For L16189 of the C>T transition in white Caucasians the values range from 11 to 15% [2, 4–6], in Koreans from about 15% [3] to 22% [7]. Except for two authors [2, 5] no explanation was given how the sequences downstream of the C-stretch had been obtained.

Parson et al. [5] describes that all data after position 16193 are confirmed by means of an independent PCR and sequence reaction in the case of a 16189 T>C transition. But once we discovered that H-strand sequencing provided less reliable results, the method of only H-strand sequencing was no longer acceptable. Therefore, we tried to resolve this problem by designing primers L16196 and L00318 which matched the poly-C-stretch and were fixed by the following complementary bases at the 3′ ends. The use of these primers for the sequencing enables the L-strand to be read correctly in the HV I as well as in the HV II regions starting from the 13th base after the C-stretch. This way the portion which cannot be sequenced in both directions is kept to a minimum. In addition, the use of the L16196 primer in one of our latest forensic cases enabled us to continue the L-strand sequencing after the sequence readings had stopped behind the homopolymeric C-tract. Hence, we were able to obtain reliable results in the range from nt16214 to nt16516 and detect rare deviations from the Cambridge reference sequence [1] in three hairs. It may seem unusual to create primers covering a homopolymeric tract. However, since they contribute to a problem unsolved to date, we want to communicate our experience and for forensic databases and casework analyses we recommend to perform mt sequencing of both the L-stand and the H-strand.

The primer sequences are: L16196 C_{10}ATG and L00318 C_{12}GCT.

Figure 1 shows two examples (1 and 2) of such an L-strand sequencing in the HV I region and similar results can be achieved in the HV II region.

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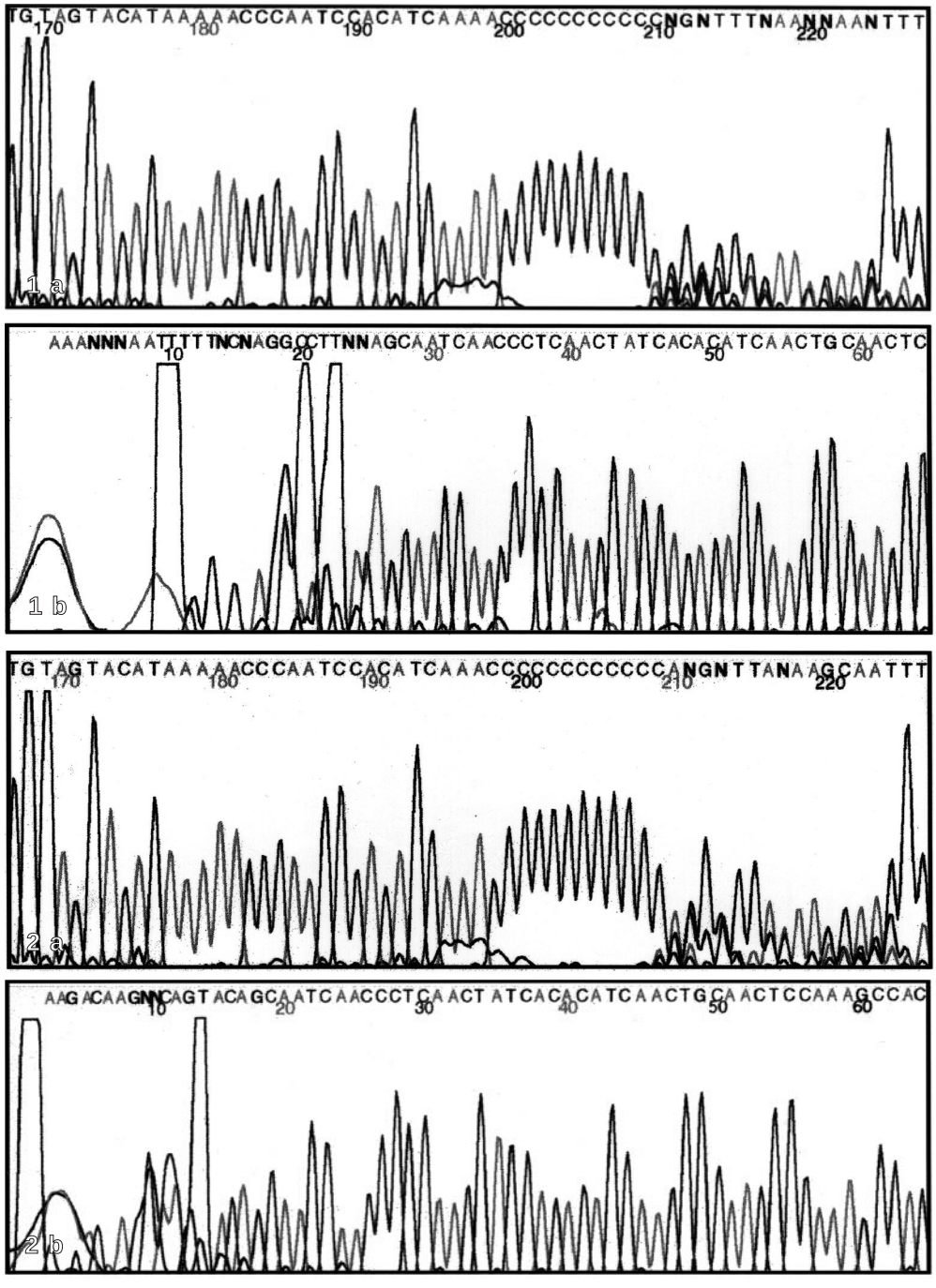

Fig. 1 The electropherograms 1a and 2a show two mtDNA d-loop sequences (nt16152 – nt16208) which are out of register due to the poly-C-stretches after sequencing using the primer L15990. The primer L16196 was then used to obtain correct sequencing results from nt16212 (lanes 1b and 2b)

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