Supplementary Note

Proof of the *in vivo* origin of allelic tetraplasmy

In this study, the following lines of evidence argue that the observed tetraplasmic allelic distribution was generated *in vivo*. First, in three individuals (p3, p4 and p6) the recombinants were detected by single fiber PCR and in two individuals (p3, p4) additionally confirmed by allele-specific PCR. Analysis of individual fibers is merely a quantification of the mutant load for each of the two genotypes in a fiber. It does not address the question whether mutants reside on the same molecule or on different ones. Thus the single fiber approach is not subject to the jumping PCR artifact[20]. Second, all allele-specific PCR reactions were carefully controlled with the use of 'triplasmic' controls obtained by mixture of DNA samples from an individual harboring a similar heteroplasmic deletion (or point mutation in the coding region) without the D-loop mutation and an individual harbouring the homoplasmic D-loop mutation. In the case of the rare D-loop mutations T16297C and del71, note that the triplasmic control presented in **Figure 1b** (t1, showing a del8426–14138 / 72C combination) was obtained under essentially the same PCR conditions. Third, in control experiments with agarose gel electrophoresis purified wild type and deleted mtDNA samples from patients p1 and p10, we were able to reproduce the allelic distribution obtained by allele-specific PCR (**Figure 2** and **Supplementary Table 1**). The purity of all agarose gel electrophoresis-separated DNA fractions was tested by multiplex PCR (**Supplementary Figure 2**). The observed contamination of individual fractions by the allelic counterpart was always lower than the measured mutation load of the D-loop polymorphism. This analysis was performed on *de novo* isolated genomic DNA from the original biopsy which excludes the possibility of a sample cross-contamination. Additionally, it is important to note that for many samples the mutations are unique, so cross-contamination is not an issue.

Thus, PCR artifacts, including jumping PCR[20] and heteroduplex formation (cf. **Supplementary Figure 3**), as well as sample cross-contamination do not represent significant problems in our approach.