

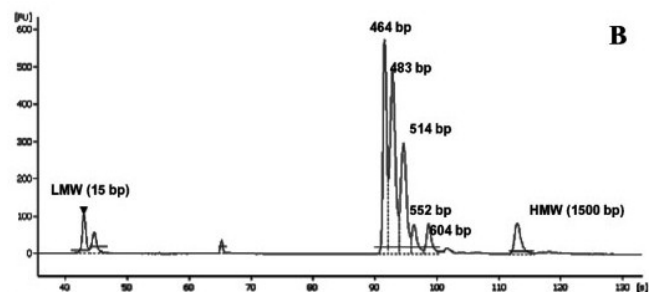
**Reference:** Alonso, A. *et al.* "Usefulness of microchip electrophoresis for the analysis of mitochondrial DNA in forensic and ancient DNA studies," *Electrophoresis*, 2006, 29, 5101-5109.

**Bioanalysis and Sample:** In this article, the authors test a commercially-available microchip electrophoresis (MCE) system with laser-induced fluorescence (LIF) detection for its utility in analyzing several types of mitochondrial DNA (mtDNA) samples. Several variants of PCR were performed (single and multiplex PCR, real-time PCR) to analyze specific regions of mtDNA (HVR1, HVR2, Cyt *b* and 16S rRNA genes) and MCE was used to quantitate and evaluate PCR products.

**Significance:** Analysis of mtDNA is important in forensics (determining the identity of an unknown person from degraded or limited samples, determining species from a hair sample) and ancient DNA studies (as mtDNA is present in high copy number in cells and less subject to degradation due to its circular structure). This paper asserts that analysis by MCE is fast, reliable, and simple and gives much lower detection limits and accurate results than those obtained by agarose gel electrophoresis.

**Technique:** In one experiment described in this paper, primers were chosen to amplify the HVR1 and HVR2 regions of mtDNA in several samples. The authors showed that MCE could be used to determine length heteroplasmy in those samples – if a sample was homoplasmic, only one size of amplicon was detected, but if a sample exhibited heteroplasmy, multiple sizes of amplicons were detected. In another experiment, both singleplex- and multiplex-PCR were used to analyze ancient (highly degraded) mtDNA samples. MCE was used to compare amplicons from these samples with amplicons from a standard sequence to determine the amounts of desired products obtained. In a third experiment, samples of mtDNA from different species were subjected to PCR in the presence of universal primers and human-specific primers designed to amplify the Cyt *b* gene. It was shown that MCE could quantify the products of these reactions, and that the human-specific primer produced a large amount of PCR amplicon from human mtDNA relative to the amount of amplicons produced from non-human mtDNA. However, the authors demonstrated that MCE has good enough detection limits to show that the human-specific primers produced a small amount of amplicon from samples of non-human mtDNA.

**Example of Results:** Electropherograms obtained from the MCE separation were used to calculate the sizes (in base pairs) of amplicons and their relative abundance. As shown in Figure 1, each separation included a 15 bp and a 1500 bp standard. The data in Figure 1 indicates that the individual exhibits length heteroplasmy in the hypervariable region 1 (HVR1), since we see amplicons of several different lengths from 464 bp to 604 bp.



**Figure 1:** electropherogram showing length heteroplasmy of an individual present in HVR1 (adapted from Alonso *et al.* 2006)

**Opinion:** In my opinion, this paper demonstrates conclusively that MCE is a sensitive and fast technique for analysis of regions of interest in mtDNA. While this work was not "cutting edge," it is important to show that newer methods like MCE can be used to replace older methods like agarose gel electrophoresis, and the authors highlighted the many advantages of MCE over agarose gels. The authors made sure to mention the advantages of using MCE in routine analysis for each of the methods they described – this will make it easier for the technique to be adopted more widely.