mtDNA TYPING IN SINGLE CELL ISOLATED BY LASER MICRODISSECTION

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The advances in microscopic instrumentation now allow direct visualization, isolation, and recovery of specific cells using approach called laser microdissection (LMD). LMD technology has been increasingly used in biomedical research applications. Relatively new applications are the use of LMD for accurate typing of forensics specimens.

For forensic purposes, contact-free microdissection and cell collection without any further manipulations seems to be an ideal approach for isolating specific cells in a contamination-free manner. In addition, sample recovery can be verified visually in a postcollection mode, allowing to inspect the cells collected microscopically.

Thus, individual cells can be separated by LMD and used directly for subsequent molecular analyses. However, the typing of laser microdissected cell preparations for microsatellite repeats indicated that problems were encountered in obtaining satisfactory STR profiles from less then 20-30 cell nuclei. Alternate technology which enables to extend further the capability of forensic typing for laser microdissected single-cell material might be the typing of the hypervariable regions of mtDNA. In this study, LMD was evaluated and an efficient, low-manipulation and non-whole-genome amplification approach was developed for forensically relevant typing of individual cells. This report describes a method for the separation of single buccal epithelial cells coupled with their downstream HVI/HVII mtDNA sequencing.

The dissections were carried out using Leica Laser Microdissection System LMD 7000 instrument (Leica Microsystems). The feasibility of LMD technology as means for single cell mtDNA analysis was assessed through two experiments.

First, various PCR buffers (GeneAmp® 10X PCR Buffer, and ABI Prism® True Allele® PCR Premix from Applied Biosystems, and Gold STR 10X Buffer from Promega Corp.) in combination with AmpliTaq Gold® DNA Polymerase (Applied Biosystem) were evaluated when applied to HVI/HVII mtDNA amplification. PCR was set up with total DNA diluted as low as one genomic DNA copy (~3.0 pg) per reaction. The product to be detected by ethidium bromide staining after two rounds of heminested PCR. For the first-round PCR we used pair of outside primers, flanking the control region including both hypervariable regions. First-round product was amplified in two separate second-round reactions (one for each of the two hypervariable regions), by using one of the outside primers and an internal primer. The results indicated that Gold STR 10X Buffer (Promega) performed best in its ability to amplify the target mtDNA molecules from highly diluted genomic DNA samples.

Then, HVI/HVII mtDNA amplification and direct sequencing was performed on preparations from single LMD-separated buccal epithelial cell. The same as above, two-round heminested amplification procedure was applied using Gold STR 10X Buffer. This proved to be advantageous for further downstream analysis, and the second-round PCR products were successfully sequenced using BigDye® Terminator v1.1 Cycle Sequencing Kit and ABI PRISM 3130 instrument (Applied Biosystems).
As a result, LMD separation of single buccal epithelial cell provided clear mtDNA sequence of the donor with no DNA contamination from exogenous source.