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Forensic applications of denaturing high-performance liquid chromatography: determination of age at death, gender determination and human identification

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1. Introduction

Denaturing high-performance liquid chromatography (dHPLC) is a new efficient tool for genomic analysis. The separation is based on the ion pair reversed phase HPLC technology in denaturing or non-denaturing conditions (denaturation is obtained by heat and by a gradient of acetonitrile).

This presentation introduces three different forensic applications of this new technology.

2. Age determination by mutation detection

Accurate estimation of age at death is a critical problem in forensic sciences. The macroscopic methods (using teeth and bones) which are commonly used, provide results with too wide a confidence interval in individuals older than 45. Several publications have shown that various mutations accumulate in mitochondrial DNA during ageing. According to these results, we are developing a new and original method to determine age at death by mutation detection. Actually, when used in denaturing conditions, dHPLC provides a rapid, automated scanning method for mutations, even when the nature and location of the mutation is unknown.

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3. Materials and methods

Mitochondrial DNA is extracted from forensic autopsy tissues (iliopsoas, liver, kidney, putamen and heart). A total of 100 individuals representing a wide age spectrum will be included in this study. Two commercial extraction kits are compared. DNA is first amplified in 13 fragments of 1–2 kb [Nucleic Acids Res. 28(20) (2000) E89]. Each PCR fragment is then digested into smaller fragments (90–600 bp) which can be separated in the dHPLC system. The separation is performed with a Transgenomic WAVE® at different temperatures to ensure the complete mitochondrial genome screening. We are studying the qualitative and quantitative differential accumulation of mutations with age among the various tissues. Our first results will be presented.

4. Gender determination

Used in non-denaturing conditions, the WAVE® can separate the two sex-specific alleles of the amelogenin locus. The separation of the X-specific and of the Y-specific allele is performed in less than 10 min without any preparation of the PCR product.

5. Human identification (separation, purification and sizing of STR)

In non-denaturing conditions, dHPLC gives strict and reproducible size-based separation of DNA fragments up to 2000 bp. This allows separation and purification of short-tandem repeats (STRs) DNA fragments. For example, the different STR alleles of HUMTH01 can be separated by dHPLC with a good resolution (>1) in less than 14 min and their size can be determined with accuracy and precision. Other STRs have been studied like the F13A01, vWa31 and FES/FPS loci.

6. Conclusion

dHPLC is a new automated and fast sizing method when used in non-denaturing conditions and is a powerful tool for mutation detection in denaturing conditions. These applications might represent a great interest in forensic genetics.