**COMPARISON OF MITOCHONDRIAL DNA SEQUENCING INSTRUMENTS FOR USE BY THE FBI'S NATIONAL MISSING PERSONS DNA DATABASE PROGRAM**

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A large number of missing person cases are unresolved in the United States. Data suggests that over 100,000 missing person cases currently exist nationwide. Recognizing the need to resolve these cases, the FBI developed the National Missing Person DNA Database Program. This program was given continuous funding by Congress in late 1999, and was implemented in early 2001. The program facilitates the association of a DNA type from unidentified remains with those from a biological relative of a missing person using the infrastructure of the Combined DNA Index System (CODIS). Since most unidentified remains are skeletal and degraded, mitochondrial DNA (mtDNA) sequencing is the initial test used to type these samples.

Reference samples from relatives of a missing person are typed using both STR analysis and mt DNA sequencing. The data are stored in the Relatives of Missing Person Index of CODIS. Additionally, all skeletal remains will be typed using mtDNA sequencing. If nuclear DNA is present in these samples, they are also typed using STR analysis. The data generated from remains are stored in the Unidentified Human Remains Index of CODIS. Comparison of these indices will be used to associate tested remains with missing person references.

Because of the demands of typing such a large number of samples, improvements are desirable in the time and/or labor involved in mtDNA sequencing. For sequencing of mtDNA, slab gel electrophoresis has been the preferred method, because a large number of samples (24 to 96) can be analyzed in parallel. Capillary electrophoreses (CE) offers an alternative approach for the separation of fluorescently-labled mtDNA fragments. The use of a CE instrument is desirable because of its ability to enable automation and decrease the run time required for sequencing. To date, CE instruments that have been available in forensic laboratories do not have the throughput capacity of slab gel systems. With the advent of multicapillary electrophoresis systems, automation and high throughput have been realized. The use of a multiple capillary electrophoresis instrument eliminates the need for gel pouring and manual sample loading, with as many as 192 samples being loaded and injected automatically.

The use and performance of the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA), a 16 capillary instrument, was evaluated for mtDNA sequencing capabilities. Performance of the ABI 3100 was compared with the slab gel-based platform currently in use in our laboratory (the ABI PRISM® 377 DNA Sequencer, Applied Biosystems) was tested. DNA sources used in this validation included: cell line, blood, saliva, bone, and hair. Hypervariable region I and II of mtDNA were sequenced using standard operating procedures. Various run times were tested using different capillary array lengths. The data presents the comparison of the three instruments, the quality and base resolution of sequences generated by each instrument, and the minimal run time required.

The results show that valid typing results can be obtained using either CE instrument, and the reliability of these CE instruments is at least equal to the performance of the ABI PRISM® 377 DNA Sequence for mtDNA sequencing. Run times for the ABI PRISM® 3100 are approximately 105 minutes (for 16 samples simultaneously) for a capillary array with a 50 cm length, and 63 minutes with an array with a 36 cm length.

**DEVELOPMENT AND USE OF PEPTIDE NUCLEIC ACIDS (PNAs) TO DETECH LOW-FREQUENCY HETEROPLASMIC POLYMORPHISM IN HUMAN MITOCHONDRIAL DNA**

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Single nucleotide polymorphisms (SNPs), insertions and deletions are commonly found in the control (non-coding) region of human mitochondrial DNA (mtDNA) and are used by the forensic community for human identification. Problems arise when the SNP is heteroplasmic (both wild-type and mutant mtDNA coexist at the same nucleotide site). If all the tissues contain the same obvious ratio of the heteroplasmic species (e.g., 50% T and 50% C), the heteroplasmy can be used as an additional source of identification. If, however, the SNP is present at a low-frequency (e.g., less than 20%) detection becomes more difficult and it is possible that two samples (i.e., one from the suspect and the other from the crime scene) will produce conflicting results, especially if hair samples are compared to blood or saliva. For this reason, the forensic community has decided that one cannot exclude a suspect based on one polymorphic difference.

To prevent this type of ambiguity, one needs a better means of detecting low-frequency heteroplasmic polymorphisms in all samples. We have utilized the unique properties of peptide nucleic acids (PNAs), to develop a simple method to block PCR amplification of the wild-type DNA while allowing the DNA containing the SNP or mutation to amplify normally. PNAs are DNA mimics with a neutral N-(2-aminoethyl) glycine backbone instead of the sugar-phosphate backbone found in DNA. A series of peptide nucleic acids (PNAs) were designed, synthesized, purified, and characterized. Thermal melting temperatures of the PNA/wild-type DNA and PNA/mutant DNA were determined to optimise the PCR binding conditions. Because PNA/DNA duplexes have higher thermal stability and sequence specificity than the corresponding DNA/DNA duplexes, PNAs can preferentially bind to their complementary DNA and prevent primer annealing, thereby blocking PCR amplification. In our case, we designed the PNA to bind to the wild-type DNA in the middle of the amplicon and distant from either the forward or reverse primers. We tested this methodology using the heteroplasmic mutation A3243G that has been associated with the mitochondrial disease Mitochondrial Ecnephalomyopathy, Lactic Acidosis and Stroke-like Episodes (MELAS). We examined DNA samples from the blood of eight MELAS patients. In the absence of PNA, the electopherograms showed little or no guanine (the mutation) at nucleotide site 3243. Adenine (the wild-type) was the predominant peak. In the presence of as little as 2μM of PNA, the predominant peak became guanine and in the most cases, the adenine peak had been eliminated. Thus, we are able to show that the use of specifically designed PNAs can be used to easily detect low-frequency heteroplasmic mutations.