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

<u>Diane K. Hancock</u> and Barbara C. Levin
National Institute of Standards and Technology, Gaithersburg, MD

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.