GENOTYPING SINGLE NUCLEOTIDE POLYMORPHISMS LOCATED ON THE Y CHROMOSOME AND IN THE MITOCHONDRIAL GENOME

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Single nucleotide polymorphisms (SNPs) are the most common form of genetic variation in the human genome. SNPs exist in approximately 1 out of every 1000 base pairs. The typing of SNPs throughout the genome can facilitate genetic mapping, disease association studies, and evolutionary studies. Recent analysis of SNPs markers located on the non-combining region of the Y chromosome provides information on tracing human migration patterns and evolution.

We are designing primer extension assays to type SNPs located on the Y chromosome as well as in the mitochondrial genome in order to evaluate their usefulness in forensic applications. The results of these primer extension reactions are being analyzed using matrix assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS) due to its inherent speed and accuracy for typing SNPs. The speed and accuracy of MALDI-TOF MS also allows rapid development of large DNA typing databases and population studies.

Because SNPs are typically bi-alleic, a greater number of these markers are needed in comparison to short tandem repeat (STR) markers for human identification purposes. Therefore, primer extension assays must be designated for improved multiplexing (typing more than one SNP per reaction) capabilities. In addition, data collection and analysis must also be optimized for high throughput evaluation of candidate SNP markers.

Our work has resulted in tools for the rapid optimization of multiplexed PCR and primer extension reactions to improve throughput for SNP analysis. Further, to date, we have compared various primer extension assays amenable to mass spectrometric analysis for SNP genotyping. The utility of MALDI-TOF MS to accurately and rapidly type samples will be illustrated through results of Y chromosome and mtDNA SNP markers, including M9, M42, M45, M89, and M96. The primer extension assays we are developing will also be compatible with other instrumentation formats such as capillary electrophoresis, fluorescence polarization and fluorescent microbeads.