# 7. Summary

In this study we describe a novel protocol for the detection of SNPs using oligonucleotide microarrays. The method relies on the allele-specific elongation of immobilized oligonucleotide primers during which a fluorescently labeled nucleotide (Cy3-dUTP) is incorporated and detected by confocal laser scanning. Cycled reactions consisting of a denaturation, annealing and elongation step are employed to increase the yield of elongated product. Using human mitochondrial DNA as a model system, we tested two different means of generating single-stranded DNA targets used in the reactions: asymmetric PCR products and exonuclease-treated PTO-modified PCR products. Both proved to be suitable in this SNP detection system. Using asymmetric PCR products, we demonstrated that a single PCR primer upstream of 9 SNPs in a 426 bp template is sufficient. 46 of 48 SNPs could be detected in this way using 3 multiplexed asymmetric PCR reactions. The disadvantage of asymmetric PCR reactions is the difficult judgement of multiplexed reactions since asymmetric PCR products appear as a smear of bands in agarose gels. The application of exonuclease-treated PTO-modified PCR products as targets is therefore advantageous. More importantly, however, we demonstrate that it is possible to type 44 out of 46 randomly distributed mitochondrial SNPs by using 5 targets of 2.5kb to 4.4kb which together cover the entire mitochondrial genome. The size limit of such targets was found to lie between 4.4kb and 5.7kb. The approach described here simplifies PCR-amplification of SNP loci, which is a major problem in transforming microarray-based SNP typing into a high-throughput method.