MULTIPLEX REAL TIME FLUOROGENIC SNP ASSAYS FOR INCREASING DISCRIMINATION OF MTDNA TESTING

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Real time fluorogenic PCR assays for allelic discrimination are widely used for single nucleotide polymorphism (SNP) testing in medical diagnostic and research settings. The TaqMan® allelic discrimination system that we report on here was run on and ABI 7700 sequence detection system, but is applicable to a variety of detection platforms. TaqMan® allelic discrimination requires amplification primers and two labeled probes for each target site. Due to the limited number of fluorescent dyes readily available, in its standard form TaqMan® can be "multiplexed" for only two sites. This is a limitation that serves to offset the advantages it presents in extreme sensitivity and ready availability as a well-developed commercial system.

We have devised and tested a modified approach to TaqMan® allelic discrimination for mtDNA typing that overcomes the multiplex limitation. This involves a multiplex amplification using the TaqMan® PCR primers for multiple target sites, followed by a second round of singleplex TaqMan® assays with the fluorogenic probes. While this is no longer a homogeneous assay, it is a simple two part assay that preserves what is by far the most important aspect of multiplexing in the forensic context: that only a single amplification needs to be performed on the casework DNA extract. We will present the design, selection strategy, and testing of 10-plex SNP assays that target sites that greatly increase the forensic discrimination of mtDNA typing when common HV1 and HV2 sequences are encountered.