ALLELE-SPECIFIC QPCR METHOD FOR SNP AND INDEL GENOTYPING BASED ON FRET

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The proposed Allele-specific q-PCR (ASQ) method for genotyping of single nucleotide polymorphism (SNP) and insertion-deletion polymorphism (InDel), based on Fluorescence resonance energy transfer (FRET). Initially named by its developers as Kompetitive Allele Specific PCR (KASP), this method is an AS-PCR variant adapted for fluorescence-based detection of amplification results. We developed a bioinformatic tool for designing probe sequences for PCR-based genotyping assays. Probe sequences are designed in both directions, and both single nucleotide polymorphisms (SNPs) and insertion-deletions (InDels) may be targeted. In addition, the tool allows discrimination of up to four allelic variants at a single SNP site. To increase both the reaction specificity and the discriminative power of SNP genotyping, each allele-specific primer is designed such that the penultimate base before the primer's 3′ end base is positioned at the SNP site. The tool allows the design of custom FRET cassette reporter systems for fluorescence-based assays. FastPCR is a user-friendly and powerful Java-based software that is freely available (http://primerdigital.com/tools/). Using the FastPCR environment and the tool for designing AS-PCR provides unparalleled flexibility for developing genotyping assays and specific and sensitive diagnostic PCR-based tests, translating into a greater likelihood of research success.