



Applications of 5-dye technology in forensic DNA typing and analysis

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In the past decade, fluorescence-based DNA detection systems have been widely used in forensic DNA analysis. Fluorescence detection methods have greatly aided the sensitivity and ease of measurement of PCR amplified short tandem repeat (STR) alleles. In the new multiplexed STR genotyping kits, fluorescent dyes are covalently coupled to primers for each locus. Fluorescence measurements involve detecting light emitted from an excited dye at a specific wavelength. Filters are used to select fluorescent dye signals at optimal wavelength ranges. In ABI PRISM® instruments, fluorescent signals are separated by a diffraction grating and projected onto a charge-coupled device (CCD) camera during data collection. Multicomponent analysis is performed using a matrix that excludes the contribution of neighboring dyes.

Earlier kits from Applied Biosystems (AmpFISTR® kits) used PCR primers with NHS-ester dyes 5-FAM™, JOE™ or NED™ and ROX™ dyes and emission spectra ranging from 522 to 607 nm. More recently, Applied Biosystems has developed a unique 5-dye technology for automated DNA fragment analysis. The introduction of the new 5-dye chemistry involves replacement of 5-FAM with 6-FAM™, JOE with VIC™ and ROX with LIZ™ and incorporation of the new PET™ dye into the existing system. The five dyes, 6-FAM, VIC, NED, PET and LIZ, expand the spectral detection range on the ABI PRISM instrumentation to 660 nm. The addition of more dyes thus enables more loci to be multiplexed into a single PCR amplification. Additionally, the new dyes will increase genotyping throughput significantly since more loci can be analyzed simultaneously in a single lane or capillary. One advantage of this arrangement is that minimal hardware changes to existing instrument platforms is necessary.

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Examples of use of this technology in Applied Biosystems kits will be presented including the Identifiler™ kit (15 loci and Amelogenin) and other kits in development (e.g. SNP detection). The performance of the 5-dye technology across the ABI PRISM 310, 377, 3700 and 3100 genetic analyzers will be presented. Also, reliable signal intensity, and software analysis compatibility with the new expanded 5-dye set will be demonstrated.