

DEVELOPMENT OF A 5-DYE DNA FRAGMENT ANALYSIS DYE SET FOR USE ON ABI PRISM® INSTRUMENTS

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Fluorescent short tandem repeat (STR) analysis is highly automatable and an informative tool for a variety of genetic assays, including human identification applications. An advantage of a fluorescent dye detection system is that DNA fragments overlapping in size range can be labeled with different dyes and thus be simultaneously detected in a single lane or injection on analysis instrumentation. Ampf/STR Profiler Plus™ and COfiler™ PCR Amplification Kits utilize 4-dye technology in the form of Dye Set F; sample PCR fragments are labeled with 5-FAM™, JOE, and NET™ and the internal-lane size standard is labeled with ROX™. Used together, these two kits allow the human identification community to amplify the 13 core STR CODIS loci from two PCR amplifications and subsequently analyze these data in two lanes or injections on ABI Prism® platforms.

While 4-dye technology provides a significant increase in throughput over the more traditional methods (e.g. radioactivity, chemiluminescence), increasing demand for genotypic information has spurred the need for even higher-throughput solutions. We describe a 5-dye system for automated fragment analysis, permitted by the development of two new fluorescent dyes for use on the ABI Prism® 377 DNA Sequencer, the ABI Prism® 310 and 3100 Genetic Analyzers, and the ABI Prism® 3700 DNA Analyzer. The two new dyes (depicted as red and orange in GeneScan® software version 3.1 or higher) were specifically designed for an expanded detection range; the orange dye has an E_{\max} of 660nm compared to 610nm for ROX™ dye. The expanded spectral range yields color separation comparable to a 4-dye set, such as Dye Set F. The 5-dye set consists of 6FAM™, VIC™, NED™, and PET™ dyes, all used to label PCR fragments, as well as a 5th dye to label the internal-lane size standard.

The 5-dye system incorporates reagents, running methods, data collection and analysis tools to enable higher throughput for a variety of applications. When applied to human identification, the 5-dye system will permit multicomponent analysis of a greater number of labeled fragments in a single lane or injection while maintaining small amplicon sizes and preserving current primer sequences. Data generated from the Ampf/STR Identifiler™ PCR Amplification Kit will illustrate the performance of the 5-dye system of the ABI Prism® 377 DNA Sequencer, the ABI Prism® 310 and 3100 Genetic Analyzers and the ABI Prism® 3700 DNA Analyzer.

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