THE APPLICATION OF PH MEDIATED SAMPLE STACKING IN THE ANALYSIS OF MULTIPLEXED SHORT TANDEM REPEATS

Frederica Crivellente and Bruce R. McCord
Department of Chemistry and Biochemistry, Ohio University, Athens, OH

A systematic evaluation of the effects on sample preparation and injection on the AmpfISTR Profiler™ system has been carried out. Dilution of amplified samples in different kinds of formamide and in water has been evaluated for the analysis of this system using a commercial capillary electrophoresis system, the PE/ABI 310.

Particular attention has focused on the effect of the sample preparation on the intensity of the signal and on the resolution. Samples were deliberately contaminated with poor quality formamide and excess concentrations of DNA and/or buffer salts. While the resolution seems not to be affected by differences in sample concentration and conductivity, the signal strength is higher in pure solvents and tends to decrease in lower quality formamide.

To improve the sensitivity of low quality samples, and on-column sample preconcentration method for capillary-based DNA analysis has been studied[1]. On column preconcentration of DNA fragments is achieved simply by electrokinetic injection of hydroxide ions. A neutralization reaction between these OH ions and the cationic buffer component $Tris^+$ results in a zone of lower conductivity, within which field focusing occurs. DNA fragments are concentrated at the front of this low-conductivity zone. Using a 44 cm long dB-17 coated capillary with HEC 2% (MW 250,000) as the separation matrix and an electric field of 340 V/cm, an improvement of sensitivity can be detected in all different types of sample preparation. This stacking method seems not to degrade the coating of the capillary at least for 20 runs. Implications of the above results will be discussed in the context of maintaining high quality DNA separations.

[1]Xiong, Park, and Swerdlow, Anal Chem 1998, 70, 3605-3611.