Forensics: Analysis of Short Tandem Repeat Loci by Multiplex PCR and Real-Time Fluorescence Detection During Capillary Electrophoresis

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1 Introduction

There are myriad genetic markers available to the forensic scientist for analyzing biological specimens found at crime scenes. Short tandem repeats (STRs), also known as microsatellites, are the most informative polymerase chain reaction (PCR)-based genetic markers for characterizing biological material [1, 2]. The STR loci are composed of tandemly repeated sequences (each of which is two to seven base pairs in length) and are highly abundant in the human genome.

The typing of STR loci is made possible by the use of the PCR. A PCR-based technology affords high sensitivity of detection. In fact, less than 1 ng of human template DNA is required for STR analysis. The specificity of amplification is such that assays designed to detect human DNA sequences only yield results with human, or higher-primate, DNA. Analyses can be performed in 1 to 2 days, and labor can be reduced substantially, because the PCR process and subsequent analyses are amenable to automation.
A feature that makes the amplification of STR loci a tremendously useful forensic tool is the relatively small size of STR alleles (generally 100–350 nucleotides). Therefore, degraded DNA samples can be successfully amplified at STR loci. Moreover, STR loci can be amplified simultaneously in a multiplex PCR. Thus, substantial information can be obtained in a single analysis with the ancillary benefits of using less template DNA, reducing labor, and reducing the chances of contamination.

In the United States, 13 STR loci are employed for typing all felons in the National DNA Databank CODIS and for attempting analyses of biospecimens found at crime scenes [3]. The 13 core loci are: CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11. The advantages of typing convicted offender samples for all 13 STR loci instead of fewer loci are (1) the augmented discrimination potential, even for mixtures, degraded samples and biological relatives; (2) fewer provisional hits when searching a data bank(s); (3) better quality control; (4) defined budgets; (5) faster response time to the police/investigator; (6) enhanced nationwide compatibility; (7) no consequence of sample loss preventing a subsequent analysis; and (8) sufficient data to obviate differences that may arise among primer sets from the different manufacturers. An ancillary benefit of selecting 13 STR loci is that manufacturers have a defined message for producing kits.

The process in evaluating candidate loci for core loci in CODIS and the subsequent selection have prompted manufacturers to develop and improve multiplex STR kits for typing up to eight or nine loci simultaneously. Currently, Perkin Elmer Applied Biosystems (Foster City, CA) produces three commercially available multifluor multiplex STR kits and the Promega Corporation (Madison, WI) produces one 8-locus STR kit (Tab. 1). Each kit provides fluorescent dye-labeled multiplex primer sets and reagents to carry out the PCR.

Given that multiplex STR kits are available, effective methods for typing the multifluor labeled STR products have been explored. Routinely, slab polyacrylamide gel electrophoresis and real time detection have been employed to separate fluor-labeled STR alleles. However, capillary electrophoresis (CE) is an attractive alternative analytical tool for the separation of DNA fragments [4]. The use of a capillary rather than a slab gel for separation of DNA fragments can augment automation, thus reducing manual efforts of the analyst and reducing reliance on the manual skills of the analyst. While many of the same parameters affecting conventional electrophoretic systems are relevant to CE [such as electroendosmosis (EEO), peak/band diffusion, joule heating, electrodispersion, interaction with the medium and surfaces, and electrophoretic separation conditions], CE has a number of desirable features. With CE, manual gel pouring and sample loading are eliminated, since loading of the sieving medium into the capillary and sample injection are achieved by automatic means. The high surface area-to-volume ratio (which enables dissipation of Joule heat) of a capillary enables electrophoretic separations to be carried out at higher field strengths. Faster separation times are thereby achieved, and resolution may be improved compared with some slab gel electrophoresis methods. Furthermore, real time detection also is performed with CE. Results are stored directly in the computer, thus facilitating subsequent data manipulations and analyses.