Separation and Purification of Short Tandem Repeat (STR) DNA Fragments Using Denaturing HPLC (DHPLC)

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Successful separation and characterization of short tandem repeats (STRs) at the F13A01 locus of human chromosome 6 was performed on the DNASep® column by Denaturing HPLC (DHPLC).

Introduction

Short tandem repeats (STRs) and microsatellites are sets of tandemly repeated oligonucleotide core sequences, which are used as genetic markers. The detection of STRs is based on the variations in length of STR-containing PCR® products. These PCR® fragments have to be purified from salts, unused dNTPs, and unincorporated primers before the automated sequencing analysis. Moreover, individual genotyping requires further separation, purification of heterozygous STR alleles from unspecific heteroduplex fragments generated by PCR® errors, and subsequent sequencing.

The WAVE® System’s capability to analyze heteroduplex formation by Denaturing HPLC (DHPLC) and to purify PCR® products for automated sequencing makes it a powerful tool in DNA profiling studies and applications.

Example

The F13A01 locus at human chromosome 6p24-25 was separated and purified with peak capture by DHPLC. Its allelic range is from 3 to 20 repeats and the repeat sequence is AAAG. The two peaks F13A (5,8) in Figure 1 are alleles 5 and 8 from the F13A01 locus from a heterozygous individual. Based on the reasoning given in Ref. 5, the heteroduplexes are detected earlier than the homoduplexes. To analyze the nature of the peak capture method for PCR® products, three fractions were captured at 18 s intervals. All fractions were re-analyzed by DHPLC to ensure only one allele was present. Figures 2A and C show peaks from the fractions containing alleles 5 and 8, respectively. The chromatogram of the third fraction (Figure 2B) demonstrates the presence of both alleles. The fractions containing the individual alleles were subjected to sequencing by capillary electrophoresis with an accuracy >96%.

Discussion

DHPLC presents a powerful and rapid method in studying STR polymorphisms. The resolution time r for the heterozygous F13A01 alleles 5 and 8 was calculated to be 1.45 by the formula:

\[ r = \frac{t_r - t_i}{W_j + W_i} \]

where \( t_r \) and \( t_i \) are the retention times of alleles one and two, and \( W_j \) and \( W_i \) are the peaks widths. A difference between the \( r \) of F13A01 and two other loci (FES/FP5 and TH01 with \( r \) of 2.04 and 1.91, respectively) with 12 bp difference between their corresponding alleles can be explained by the size of the fragments and to a lesser extent the base composition differences. However, the PCR® product of F13A01 is 70% AT and has a low r value due to the corresponding interaction of the base pairs with the column substrate. All fractions were analyzed successfully by capillary electrophoresis sequencing without any ad-
ditional sample preparation (see Application Note No. 104, “PCR* Amplification and Automated Sequencing of DNA Fragments Isolated with the WAVE® System”).

**Experimental Methods**
Genomic DNA was extracted from human blood with the Puregene DNA Isolation Kit (Genetix Systems, Inc., Minneapolis, MN). The F13A01 tetrameric loci were amplified with primers developed by the Forensic Service of the British Home Office for use in forensic casework. PCR* reactions were performed with Taq Gold™ (Perkin Elmer, Foster City, CA) with the following program: 10 min at 95°C, 28 s at 94°C, 1 min at 54°C – 2 min ramp, 1 min at 72°C, 10 min at 72°C, 4°C soak. The DHPLC was carried out with a gradient mixture of buffer A: 0.1 M triethylammonium acetate, i.e., TEAA (available from Transgenomic) and buffer B: 0.1 M TEAA/25% acetonitrile at a flow rate of 0.3 mL/min.

**Conclusion**
Successful analysis of the STR locus alleles with DHPLC makes this technique a useful typing method. The collected DNA peaks could be sequenced directly, which simplifies the process of data acquisition.³

**References**

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* PCR is a process covered by patents issued and applicable in certain countries. Transgenomic does not encourage or support the unauthorized or unlicensed use of the PCR process.

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