AUTOMATED LASER FLUORESCENT ANALYSIS OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS AND SHORT TANDEM REPEATS

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Genetic typing of Variable Number of Tandem Repeat (VNTR) loci is today feasible by the use of fluorescent primers in otherwise typical PCR reactions, and by subsequent laser-detection of amplified products during the electrophoretic run. These facilities, derived from procedures now currently used in automated DNA sequencing, allow for rapid and reliable typing of a variety of Amplified Fragment Lenght (AmpFLP) and Short Tandem Repeats (STRs) loci. Their adoption is having a remarkable role in speeding up procedures of forensic investigation (individual identification and paternity tests). We briefly report on our experience in typing VNTRs by a fixed argon laser-beam sequencer (A.L.F,Pharmacia) (1).

MATERIALS AND METHODS

Amplimers were synthesized on a 391 PCR-Mate (ABI) using a slightly modified cycle to label the 5'-end of one primer of any given pair by a fluorescein-amidite (FluorePrimeTM, Pharmacia). Genomic DNA was used to amplify the following loci: D1S80 (2), HUMACTBP2 (3), HUMTH01 (4), MIT-MH26 (5). Annealing temperatures were slightly adjusted to account for the presence of the fluorochrome molecule. Amplified products (0.5 to 3 microliter) were denaturated and dispensed in the vertical wells of the A.L.F. gels (6% T acrilamide, 7 M urea). Running conditions were 1500 V, 35 mA and 30 W and were administered for 6 hours to allow a simultaneous characterization of the four loci. Home-made DNA allelic ladders, composed of coamplified VNTR or STR alleles from different genotypes in pools, helped to control the gel resolving power and the final genetic typing. To identify alleles, comparison of adjacent lanes and occasional admixtures of PCR products were performed. Multiplexing of HUMTH01, MIT-MH26 and D1S80 was obtained by simultaneous gel separation of the three systems in the same lane. No attempt to amplify different systems in the same PCR cycle was performed.

RESULTS AND DISCUSSION

Figure 1 shows some typical chromatograms produced by the A.L.F. apparatus. For D1S80, a standard collection of 15 distinct fluorescent fractions was used, defining as many

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alleles differing by 16 bp. For the other loci, an allelic difference of 4 bp is always met. Noteworthy, a correct separation and typing of all systems is possible in one standard experiment, irrespective of the basic difference in size and repeat unit. A comparison between the laser fluorograms and PAGE/silver procedures demonstrated the superior sensitivity and reliability of the automated procedure and its versatility. However, the use of a single fluorescent molecule made it possible "multiplexing" (i.e.: analysis in the same well) of only these systems with non-overlapping sizes. The A.L.F. resolving power clearly emerges by the fact that the so called 10-1 allele in the HUMTH01 system was easily typed (this fragment variant differs by a single base pair from the nearest allele). Experiments with age-degraded DNA samples showed that laser fluorence sensitivity is higher (by a factor of about 100) than the PAGE/silver procedure. The automated typing protocol is presently employed in our routine of paternity cases and its application to criminal analyses is planned.

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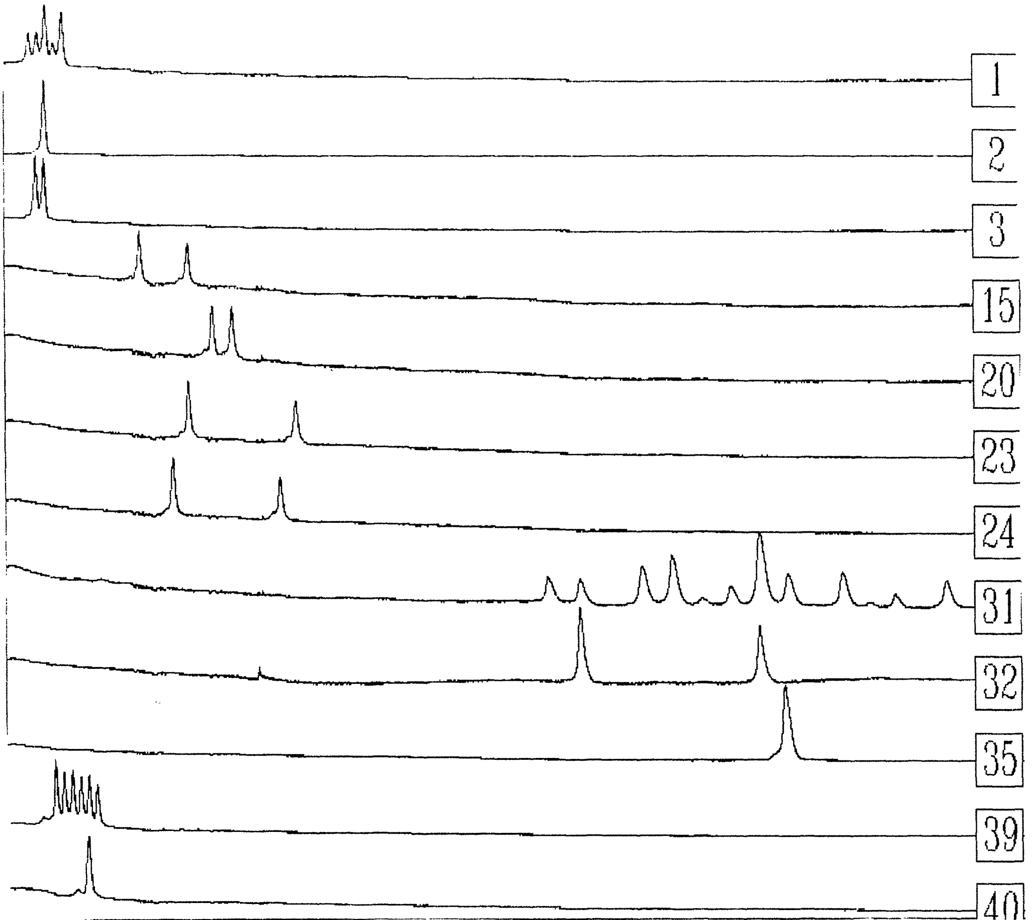

Figure 1
A typical chromatogram produced by the A.L.F. apparatus is shown. The simultaneous detection of four loci is achieved in a six hours run. In the first three lanes an examplification of the commonest allels of MITMH26 system is reported. In the subsequent lanes (n. 15, 20, 23 and 24) some HUMACTBP2 fragments are detected. A D1S80 fluorescent allelic ladder has been electrophoresed in lane number 31, while two amplified products for the same system are present in the adjacent lanes (n.32 and 35). The last two chromatograms show the allelic ladder and an homozygous individual for the HUMTH01 system.

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