COMPARISON OF MANUAL AND AUTOMATED DETECTION FOR STRs ANALYSIS.

P. Montagna, A.L. Pezza, S. Tavano and A. Spinella.
Servizio Polizia Scientifica, Sez. Indagini Biologiche, Roma, Italia.

Introduction

The short tamdem repeat (STRs) loci are ideal for identification in forensic investigations. Being STRs repeated in a tandem fashion, highly polymorphic, extremely abundant and evenly distributed in the genome (1). PCR amplification has improved the sensitivity of their DNA profiling (2). Here we report comparative results from the standard approch to microsatellite analysis (3) and automated fluorescence based technology (4) to detect three different microsatellite loci.

Materials and Methods

Human genomic DNA was prepared from peripheral blood and dry bloodstains by standard methods involving cell lysis, proteinase K digestion and ethanol precipitation. Three microsatellite loci were used in this study: ACTBP2 (Polymeropoulos, M. M. et al.), MBP (Polimeropoulos, M.M. et al.) and HUMTM01 (Edwards, et al.).

All oligonucleotide primers used were syntesized using a Model 380 DNA Synthesizer (Applied Biosystems, Foster City, CA). For the automated detection a fluorescent dye (JOE, FAM and TAMRA) was attached to the 5'- end of the forward PCR primer used for amplifying each locus using Aminolink 2 (Applied Biosystems, Foster City, CA) and HPLC purified.

We used an internal lane size standards consisted of a PstI digest of Lambda phage, labelled with a fourth red dye (ROX) by a legation method (5). Nonfluorescent primers were synthesized using the same sequences.

PCR were carried out for fluorescence primers for 25 cycles with 25-50 ng of DNA in 50 µl reaction volume. Each locus was initially amplified in a single reaction. Then MBP and ACTBP2 loci were amplified in the same tube with fluorescent primers labelled with JOE (green) and FAM (blue) respectively. HUMTM01 was labelled with TAMRA-dye (yellow) and amplified with single PCR.

For gel analysis with fluorescent amplification products, 0.5µl of the internal lane standards and 2µl of PCR reactions for multiplex or 1µl for simple locus were combined with 3µl of deionized formamide. Were electrophoresed in a 6% Acylamide gel (BIORAD) with 8M urea in 1x TBE. Gels were electrophoresed for 6 hr at 30W constant power on an Applied Biosystems automated DNA sequencer model 373A. The size of PCR products is determined automatically and precisely by GENESCAN 672 software (Applied Biosystems, Foster City, CA) using the labelled size standard in each lane.

Non fluorescent amplified STRs loci were analyzed on horizontal polyacrylamide gels according to the F.B.I. protocol.

Gel separation was performed by horizontal discontinuous PAGE (7% to 10%). The gels were cast on gelbond using the Flap technique. The gels were cross-linked with piperazine diacrylamide (29:1) prepared in 0.12M Tris-Formate buffer, pH 9.0.The trailing buffer consisted in 2x tris Borate, pH 9, 0,28M.Electrophoresis was conducted at 20mA, electrode distance 2 cm.Following electrophoresis, the gels were stained with silver (Allen, 1989).

RESULTS:
Comparisons of allelic profiles obtained from conventional electrophoresis (3) with results from GENESCAN 672 Software have indicated no significant difference. Indeed, when we compare allele lenghts derivated from technique using allelic ladder standard and results from automated fluorescent analysis some difference has been found on allele designation.

Our prelyminary results (Fig. 1) indicate that high accurancy and precision can be obtained with GENESCAN 672 Software. The size of PCR products is determinated automatically by the software using the labelled size standard in each lane. It is the best way to provide lane to lane reproducibility and a precise size calling. For forensic area to obtain valid and reliable results from a variety of biological sample is an important parameter. The amplification from old bloodstains and a gel separation with standard method can displayed differences in the size of alleles in each STR locus. In this condition we observed an apparently different sized allele which do not match with an allelic ladder. In this experiment an inclusion or exclusion is determinated by profile comparison. The areas in a ladder where there in no an allele for comparision, can be assigned an appropriate allele size. This is significant in microsatellite analysis where alleles can differ in size by as little as one or two base pairs.

Futhermore, this technology increases the number of the markers that can be genotyped in each gel lane. We have multiplex different colors and different size ranges in the same lane (Fig. 2).

Finally, we believe that the advantage to use this technology which is based on three available fluorescent dyes, is the high sensitivity and resolution that should address the realistic amount of DNA in forensic nature.

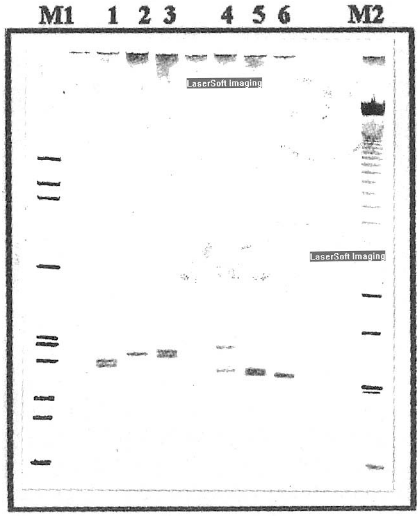

Figure 1. Horizontal gel page, silver-stained STR (ACTBP2).

M1, M2	Molecular weight standard.
Lane 1	Amplified DNA blood suspect 1.
Lane 2	Amplified DNA bloodstain.
Lane 3	Amplified DNA blood suspect 2.
Lane 4, 5, 6	Mendelian inheritance.

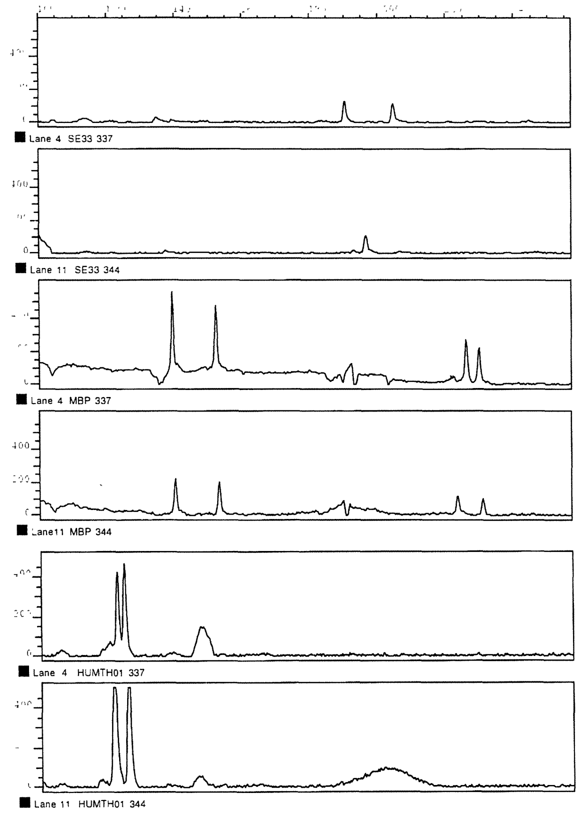

Fig. 2: Electrophoretogram display of PCR products of three STRs run in the same gel lane and distinguished by three different dyes.

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