STR TYPING WITHOUT DNA EXTRACTION USING AN INFRARED-BASED NON-RADIOACTIVE AUTOMATED DNA SEQUENCER

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ABSTRACT: A LI-COR Model 4000 automated DNA sequencer using high sensitivity infrared (IR) fluorescence technology was used to detect STR allele patterns from bloodstains and simulated forensic samples using *Tth* polymerase. Two different amplification strategies were used for labeling. Multiplexing of three primer pairs in a single PCR amplification was accomplished using *Taq* polymerase. Typing of STR alleles was also achieved using a *GenePrint*™ STR System (Promega) for various forensic-like specimens. Genotyping of the O allele of the human ABO blood group locus as well as the gender differentiating amelogenin locus was also performed using both strategies. This system combines IR fluorescence chemistry and laser technology thus eliminating the need for radioactivity and the gel handling required with some methodologies. STR alleles are displayed as autoradiogram-like images during the run and can be computer analyzed.

MATERIALS AND METHODS: The procedure for collection, amplification and gel electrophoresis of samples is according to the protocol described by Roy et al (1995). Bloodstains were collected from volunteer donors on two types of sterilized fabrics: 100% cotton and a cotton + polyester mixture. Approximately 1 mm of bloodstained thread was placed in an autoclaved reaction tube and directly amplified using *Tth* polymerase and a high temperature incubation to extract DNA from blood cells. To validate the results from bloodstain analysis, STR alleles were also amplified using Chelex extracted DNA from bloodstains, saliva, hair roots, semen, vaginal fluid and other simulated forensic samples. Electrophoresis and detection were performed using a LI-COR Model 4000 Automated DNA Sequencer. DNA for multiplex reactions was purified using a Genomix blood DNA extraction kit from Washington Biotechnology (Bethesda, MD).

Two strategies for automated IR fluorescence detection of PCR products from polymorphic repeat regions were utilized: 1.) One of the PCR primers had a 19 base extension at its 5' end with the sequence 5'-CACGACGTTGTAAAACGAC-3'. This sequence is identical to an IR-labeled universal M13 Forward (-29) primer which is included in the amplification reaction. During PCR the tailed primer generates a complementary sequence to the M13 primer which is subsequently utilized for priming in the amplification reaction thereby generating IR-labeled PCR products. 2.) A limited quantity of an IR-labeled deoxynucleotide (dATP) was included in the amplification reaction. During DNA synthesis the polymerase occasionally incorporates a labeled molecule into the growing DNA chain thus producing a PCR product internally labeled with IR fluorophore.

STR loci analyzed included the following: ACTBP2, D2S436, D18S535, D20S470 & HUMTHO1. Primers for the amelogenin locus were derived from sequence described by Sullivan et al (1993) and synthesized by Genosys (The Woodlands, TX). For the internal labeling strategy, ameloginin amplification primers were also obtained from a *GenePrint*™ STR System (Promega Corporation; Madison, WI). Multiplex primers for CSF1PO, TPOX and THO1 loci were also obtained from *GenePrint*™ STR Multiplex Kit. Primers for the O allele of the ABO locus were obtained from Genosys using the sequence described by Crouse and Vincek (1995). Molecular weight markers primarily utilized consisted of one lane of a standard sequencing reaction.

CONCLUSIONS: STR loci were detected directly from dried bloodstained fabric using *Tth* polymerase without time consuming DNA extraction and purification. Multiplexing with either three tailed primers or the *GenePrint*™ Multiplex STR System with internal labeling using *Taq*

polymerase can be achieved in one reaction tube using extracted DNA. Automated determination of STR polymorphisms, gender and the O allele can be performed with this methodology. The actual raw data is visualized during gel electrophoresis and stored automatically into a database.

REFERENCES:

1. Crouse C, Vincek V (1995) Identification of ABO alleles on forensic-type specimens using rapid-ABO genotyping. Biotechniques 18: 478-483.
2. Roy R, Steffens DL, Gartside B, Jang GY, Brumbaugh JA (submitted for publication) Producing STR locus patterns from bloodstains and other forensic samples using an infrared fluorescent automated DNA sequencer. Journal of Forensic Sciences.
3. Sullivan KM, Armando M, Kimpton CP, Gill P (1993) A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechn 15: 636-639.

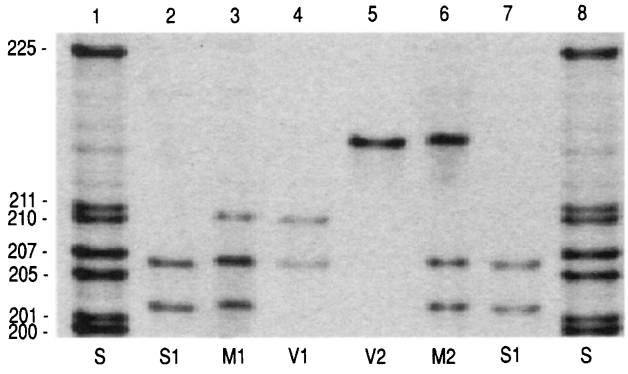

Figure 1. Allelic profiles for the HUMTHO1 locus from individual and mixed bloodstains directly amplified using *Tth* polymerase and tailed primer. Lanes 2 and 7 represent bloodstains from a suspect (S1) amplified from 100% cotton and cotton-polyester fabric, respectively. Lanes 4 and 5 represent bloodstains collected on 100% cotton from two victims (V1 and V2, respectively). Lane 3 (M1) represents a mixture of blood from S1 (bloodstains on 100% cotton) and V1. Lane 6 (M2) represents a mixture of blood from V2 and S1 (bloodstain on cotton-polyester fabric). Lanes 1 and 8 are molecular weight size standards (S) shown to the left of the image (in base pairs).

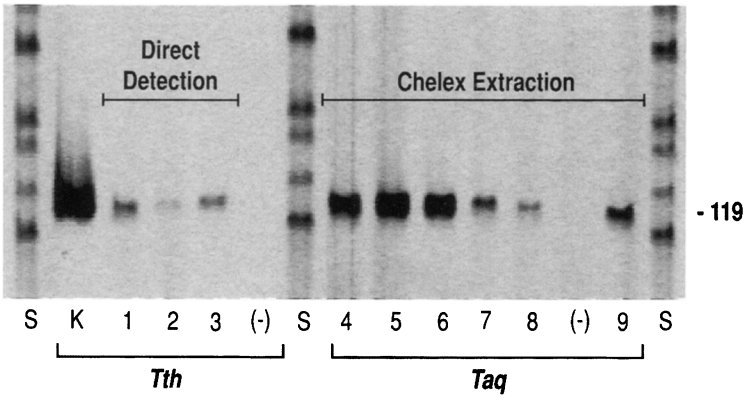

Figure 2. Amplification of O alleles (119 base pairs) from AO, BO and OO genotype individuals using tailed O primer and either *Tth* or *Taq* polymerase. K = K562 DNA (Promega). Lanes 1, 2 & 3 are direct detection using a high temperature incubation from bloodstained thread (100% cotton) from O, A & B phenotypic individuals, respectively. Lanes 4, 5 & 6 are Chelex extracted DNA from bloodstained thread (100% cotton) from three O individuals. Lanes 7 & 8 are Chelex extracted DNA from bloodstained cotton-polyester from A and B phenotypes, respectively. (-) = negative control. S = Molecular weight size standard.

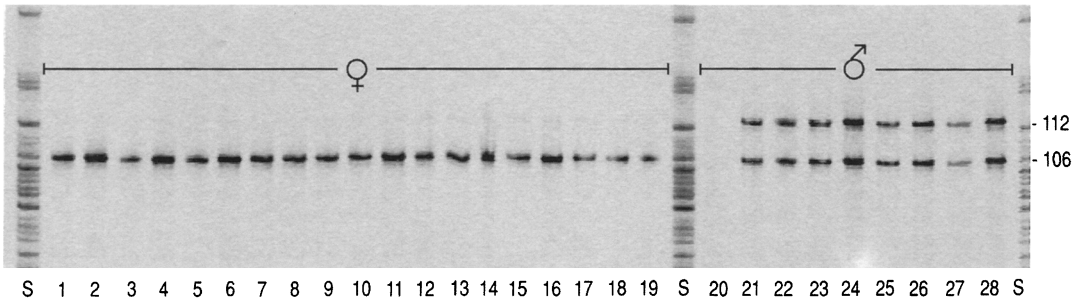

Figure 3. Amplification of extracted DNA from simulated forensic samples at the amelogenin locus using *Taq* polymerase and internal label. Blood (l), saliva (2), vaginal swab (3), vaginal wipe (4), hair (5), toe nail (6), earring (7), cigarette butt (8), toothbrush (9), head band (10), underarm (11), shed skin (12), earring (13), cigarette butt (14), rain soaked cigarette butt (15), vaginal swab (16&17), eyebrow (18) and saliva (19) samples from a female volunteer were analyzed. Negative control (20). Blood (21), semen-cloth (22), semen-paper (23), saliva (24), toe nail (25), toothbrush (26), cigarette butt (27) and hair (28) samples were from a male volunteer. S = Molecular weight size standard. Allele sizes are shown on the right of the figure.

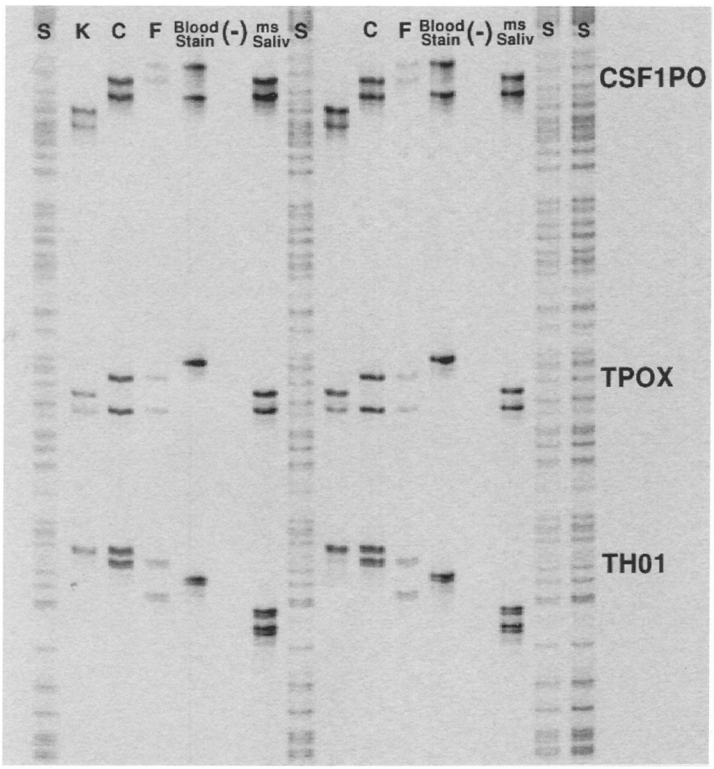

Figure 4. Detection of three polymorphic STR loci using purified DNA from a father (F) and child (C); and using Chelex extracted DNA from bloodstains and saliva using the *GenePrint*™ STR Multiplex System and internal label. K = K562, (-) = Negative Control, ms Saliv = saliva from an individual, S = Molecular weight size standard. STR loci are designated on the right of the image.