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Producing STR Locus Patterns from Bloodstains and Other Forensic Samples Using an Infrared Fluorescent Automated DNA Sequencer

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ABSTRACT: Short tandem repeat (STR) analysis is increasingly being used in forensic case analysis because of the large number of STR loci in the human genome and their highly polymorphic nature. An automated DNA sequencer using high sensitivity infrared (IR) fluorescence technology was used to detect STR allele patterns from simulated forensic samples. The amplification strategy used a 19 base pair extension on the 5′ end of one of the PCR primers. This sequence is identical to the sequence of a universal M13 Forward sequencing primer which is included in the amplification reaction. Allelic bands were detected by incorporation of the M13 primer-fluorescent dye conjugate into PCR products thus eliminating the need for direct conjugation of fluorescent dye to individual STR primers.

By using an IR-based automated DNA sequencer and Tth DNA polymerase, polymorphic STR alleles were detected on-line rapidly and efficiently from bloodstains using only a high temperature incubation to extract DNA from blood cells. Five STR loci were also amplified using Chelex extracted DNA from simulated forensic samples. Multiplexing of three primer pairs in a single PCR mixture for amplification was accomplished using Taq polymerase.

This system combines IR fluorescence chemistry and laser technology thus eliminating the need for radioactivity and the gel handling required with silver staining and fluor detection systems. Real-time detection permits immediate visualization of the data and STR alleles are displayed as familiar autoradiogramlike images that can be analyzed by computer. By loading a 64 lane gel twice and multiplexing with three primer pairs, forensic scientists can type at least three loci from 120 samples in one day.

KEYWORDS: forensic science, DNA, short tandem repeats, polymerase chain reaction, Tth polymerase, infrared, automated DNA sequencer, fluorescence, ACTBP2, D2S436, HUMTHO1, D20S470, D18S535

Highly polymorphic short tandem repeat (STR) loci, or microsatellites, offer great promise for forensic analysis, paternity testing and gene mapping (1). STR loci consist of simple tandemly repeated sequences 1–6 base pairs in length. Similar to the variable number of tandem repeats (VNTR) loci, or minisatellites, many STR loci exhibit length polymorphism among individuals due to variation in the number of repeat units. STR loci are found in large numbers throughout the human genome and are usually found approximately every six to ten kilobases (2).

Since forensic samples are often degraded and limited in quantity, they may not always be suitable for analysis by conventional restriction fragment length polymorphism (RFLP) analysis. Amplification of such samples by the polymerase chain reaction (PCR) method may yield information as to the genetic identity of individuals. Since the STR loci have small allele sizes (generally less than 300 base pairs) they can be amplified easily by PCR and degraded samples are more amenable to analysis. The alleles can be separated by polyacrylamide gel electrophoresis, which is capable of resolving amplification products that vary in size by a single base, thus allowing accurate allele identification and bypassing the problems associated with continuous allele distribution models necessary for VNTR analysis (3,4). STR DNA profiling can be completed in a few days rather than several weeks as necessitated in RFLP analysis. STR analysis is currently being used for forensic identification (5,6).

This research was undertaken to determine if an automated online detection system using infrared (IR) laser irradiation could be used to detect STR alleles from bloodstains with mininal DNA extraction (such as, a high temperature incubation). The near infrared region of the spectrum (700–3000 nm) provides detection advantages over visible wavelengths due to its low background and high sensitivity (7). The use of a fluorescent reporter group eliminates the need for radioactivity and allows automated collection of data and analysis. This system does not require postelectrophoretic DNA staining or gel processing as does silver staining and other fluor detection systems, thus simplifying the analysis protocol and reducing the chance of failure.

To validate the results from bloodstain analysis, STR alleles were also amplified using Chelex extracted DNA from bloodstains, saliva, hair, and other simulated forensic samples from one individual following the same protocol. Since the majority of forensic serology cases are sexual assaults, semen and vaginal swab samples were included in this analysis. Multiplexing (8,9) using Taq polymerase and at least three primer sets in a single amplification reaction was also conducted on purified DNA samples to increase throughput and allow typing of numerous genetic loci in a minimal amount of time.

Materials and Methods

DNA Samples

Bloodstains were collected from volunteer donors on two types of sterilized fabrics—100% cotton and a cotton plus polyester

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mixture (cotton-polyester). A drop of blood was also smeared on a sterilized glass slide from each donor. Approximately 1 mm or less of the bloodstained thread was cut from each stain and placed in an autoclaved reaction tube. The tip of a sterilized needle was used to pick up a flake of dried blood from the slide and place it in an autoclaved reaction tube. Blood, saliva and hair root samples were collected from various donors as bloodstains, buccal swabs and pulled hair samples. Semen, vaginal swabs and other simulated forensic samples were collected from volunteers. All samples were stored at room temperature for at least one month prior to STR typing to simulate the time delay between occurrence of the crime and discovery, processing and/or analysis of the evidence which is often encountered in forensic casework in Nebraska.

DNA Extraction

For STR typing requiring extracted DNA the bloodstains, buccal swabs, hair roots, semen, vaginal swabs, and other simulated forensic samples (such as, perspiration, cigarette butts, toe nails, etc.) were subjected to Chelex extraction as described (10). DNA for multiplexing reactions was obtained from a nuclear family (laboratory personnel). Blood samples were taken from each family member (with assistance from Dr. Michael A. Schmidt) and genomic DNA was prepared using a Genomix Blood DNA extraction kit from Washington Biotechnology (Bethesda, MD). DNA was quantitated by UV absorbance spectrophotometry (at 260 nm).

Amplification Strategy

Three strategies for automated IR fluorescent detection of PCR products from polymorphic repeat regions can be used.

1. Amplification primers that flank the repeat region can be synthesized so that one primer is labeled at its 5′ end with an infrared fluorescent molecule. Amplification using these primers will directly label the PCR product.
2. One of the PCR primers can have 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 (Fig. 1) (11) 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 (Fig. 2).
3. A limited quantity of an IR-labeled deoxynucleotide (such as dATP) can be included in the amplification reaction. During DNA synthesis the polymerase will occasionally incorporate a labeled molecule into the growing DNA chain thus producing PCR product internally labeled with the IR fluorophore.

The authors elected to use the second strategy because it makes use of readily available IR-labeled universal sequencing primers, which eliminates the need for a labeled PCR primer for each locus analyzed. New loci can be analyzed by synthesizing a primer containing the 5′ tail rather than subjecting the oligonucleotide to labor intensive fluorophore attachment. Studies are currently underway using the third strategy to assess the quality of internally labeled amplification products. Figure 2 illustrates the amplification strategy for a single locus. Three primers are used in the STR

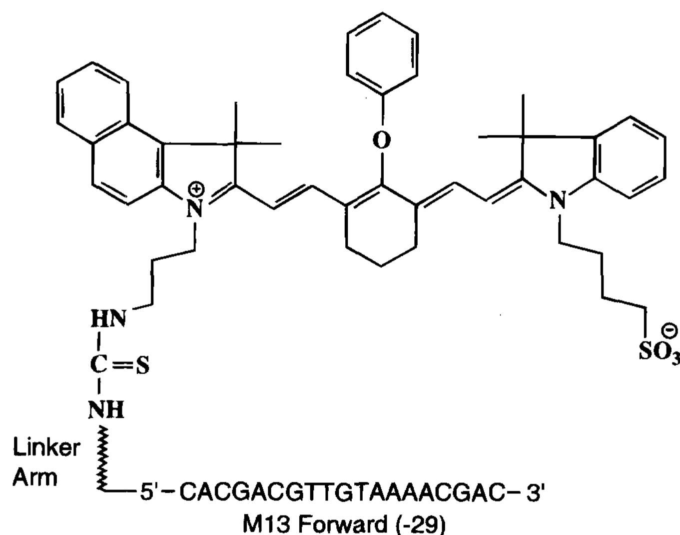

FIG. 1—*Universal M13 Forward (−29) primer labeled with infrared fluorescent dye IRD41.*

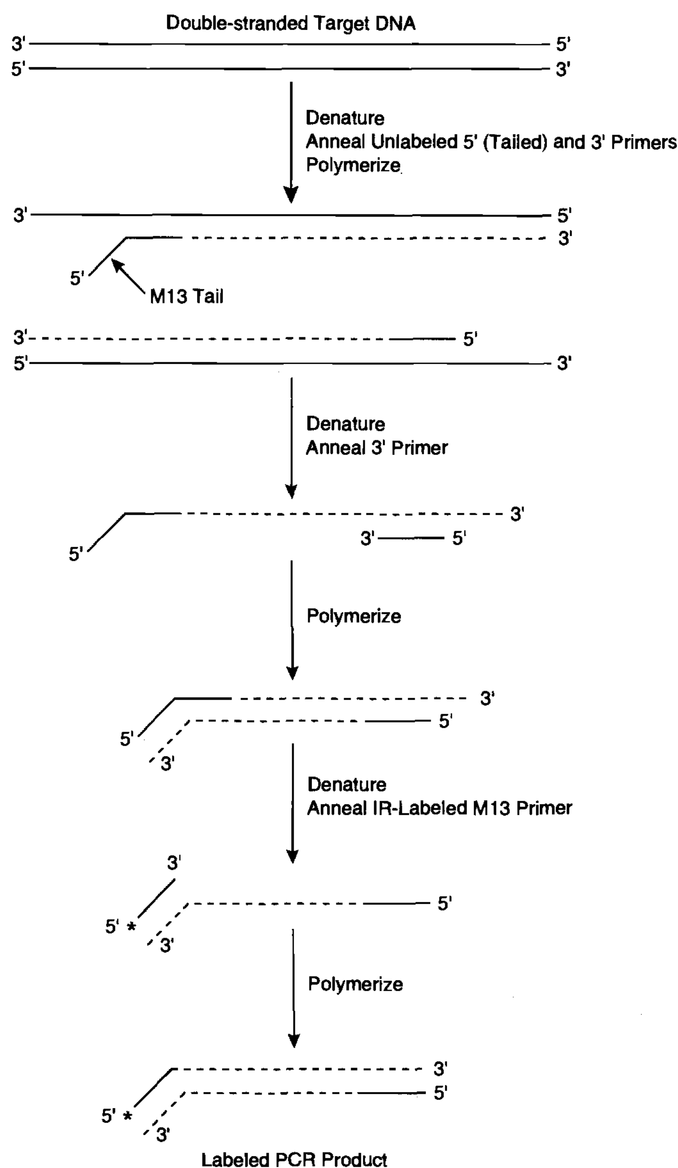

FIG. 2—*Amplification strategy for a single STR locus. Only the molecular forms involved in generating the labeled PCR product are shown.*

reaction: 1) a tailed primer at the 5′ end of the target region; 2) a non-tailed primer at the 3′ end; 3) the M13 Forward primer tagged with infrared dye (12) for labeling the resulting amplification products. For multiplexing, two additional unlabeled amplification primers (one containing the 5′ M13 tail) are added to the reaction for each additional locus to be analyzed.

Amplification Primers

Five tetrameric STR loci were chosen for amplification. All forward primers contain the 19 base universal M13 forward sequence at their 5′ end (italicized). Primer sequences are as follows:

1. Locus Name: ACTBP2 (13)
   Forward Primer: 5′-*CACGACGTTGTAAAACGAC*ACATCT-CCCCTACCGCTATA-3′
   Reverse Primer: 5′-AATCTGGGCGACAAGAGTGA-3′
2. Locus Name: D2S436 (14)
   Forward Primer: 5′-*CACGACGTTGTAAAACGAC*GATATG-GGAGCAACATGAGC-3′
   Reverse Primer: 5′-GGAATCAACTTTCAGTATAAACCC-3′
3. Locus Name: HUMTHO1 (15,16)
   Forward Primer: 5′-*CACGACGTTGTAAAACGAC*GTGGG-CTGAAAAGCTCCCGATTAT-3′
   Reverse Primer: 5′-ATTCAAAGGGTATCTGGGCTCTGG-3′
4. Locus Name: D20S470 (14)
   Forward Primer: 5′-*CACGACGTTGTAAAACGAC*CCTTG-GGGGATATAGCCTAA-3′
   Reverse Primer: 5′-TGAGTGACAGAGTGATACCATG-3′
5. Locus Name: D18S535 (14)
   Forward Primer: 5′-*CACGACGTTGTAAAACGAC*TCATGT-GACAAAAGCCACAC-3′
   Reverse Primer: 5′-AGACAGAAATATAGATGAGAAT-GCA-3′

Primers for the ACTBP2 locus were synthesized by Bio-Synthesis, Inc., (Lewisville, TX). HUMTHO1, D2S436, D20S470, and D18S535 primers were obtained from Research Genetics, Inc., (Huntsville, AL). Primer sequences for the D2S436, D20S470, and D18S535 loci were obtained from the work of Dr. Jeffrey Murray at the Cooperative Human Linkage Center (Iowa City, IA). The IR-labeled M13 Forward (−29) primer (11) was obtained from LI-COR, Inc. (Lincoln, NE).

Amplification Protocol for Dry Bloodstains

All STR loci were amplified using PCR methodology (17). All reagents were from Boehringer Mannheim (Indianapolis, IN) unless otherwise designated. Approximately 1 mm of bloodstained thread or a dry flake of blood was used from each stain. A high temperature incubation prior to thermal cycling ("heat-soaked PCR"), which has been shown to enhance amplification performance of forensic samples was used (18). This procedure serves to extract the genomic DNA from the cellular samples with a minimal expense of labor and reduces the possibility of mislabeling and contamination during multi-step DNA purifications. A "hot start" PCR procedure to enhance specificity was also used (19,20).

I. The following were combined in a 0.5 mL microcentrifuge tube and placed on ice:

A. Bloodstained fabric or dried blood

B. 10 × PCR buffer (100 mM Tris-HCl, pH 8.9; 1 M KCl; 15 mM $MgCl_2$; 500 μg/μL BSA; 0.5% (V/V) Tween 20 — 1.1 μL
C. Forward primer (with M13 tail), 5.0 pmol/μL — 1.1 μL
D. Reverse Primer, 5.0 pmol/μL — 1.1 μL
E. Sterilized distilled water to bring total volume to - 8.0 μL

II. The tubes were then placed in a Perkin Elmer GeneAmp PCR System 9600 and subjected to the following incubation:

85°C for 10 minutes
92°C for 20 minutes

III. During the high temperature incubation a second mixture for each reaction was prepared:

A. 2 mM dNTP mix (containing 7-deaza-dGTP) (Boehringer Mannheim, Indianapolis, IN) — 2.2 μL
B. IR-labeled M13 Forward (−29) primer (1 pmol/μL) (LI-COR, Inc., Lincoln, NE) — 0.3 μL
C. Tth DNA polymerase (5 Units/μL) (Boehringer Mannheim, Indianapolis, IN) — 0.2 μL

The final volume of this second mixture is 2.7 μL

IV. 2.7 μL of the second mixture was added to each reaction tube (Step II; at 92°C) in the thermocycler. The amplification was continued as follows:

A. 90°C for 5 min
B. 94°C for 40 s
C. 67°C for 60 s

Steps B and C were repeated for a total of 10 cycles.

D. 94°C for 20 s
E. 62°C for 45 s

Steps D and E were repeated for a total of 27 cycles. This was followed by a 10 minute incubation at 72°C and a final 4°C soak. When thermocycling was completed 5 μL of loading buffer (Amersham Life Sciences; Arlington Heights, IL) was added to each reaction tube making the total volume approximately 16 μL. In most cases amplified products were serially diluted 1:11 five times (final dilution of approximately 1:166,000) with loading buffer. The materials and methods for gel electrophoresis and sample loading are discussed within the Electrophoresis section.

Amplification of Extracted DNA

DNA extracted from bloodstains, buccal swabs, hair roots, semen, vaginal swabs, etc. by the Chelex procedure (10) was amplified as described in the protocol. Instead of bloodstained fabric or flakes of dried blood, 2 μL of extracted DNA was added to the first reaction mixture. This quantity has been empirically determined to produce acceptable amplification results. The volume of extracted DNA can be adjusted in subsequent amplifications for optimal results.

Multiplex STR Protocol

A volume of 2 μL genomic DNA (1 ng/μL) was used in each amplification reaction containing the following:

A.	PCR Buffer (containing 1.5 mM $MgCl_2$)	2.0 μL
B.	Each PCR primer (5 pmol/μL)	0.5 μL
C.	IR-labeled M13 Forward (−29) primer (1 pmol/μL)	0.7 μL
D.	2 mM dATP, dCTP, 7-deaza-dGTP and dTTP	1.0 μL
E.	TaqStart Antibody (Clontech; Palo Alto, CA)	0.3 μL
F.	Taq polymerase (1 Unit/μL)	0.3 μL
G.	Sterile distilled water to bring total volume to	10 μL

The reactions were cycled as follows:

A. 93°C for 5 min
B. 94°C for 20 s
C. 67°C for 60 s

Steps B and C were repeated for 10 cycles.

D. 94°C for 20 s
E. 62°C for 60 s

Steps D and E were repeated for 26 cycles. At the end of the amplification 5 μL of loading buffer was added to each reaction followed by a 1:10 dilution.

Electrophoresis

Only molecular biology grade reagents and chemicals were used when available. Gel electrophoresis and visualization of the STR alleles were accomplished using a LI-COR Model 4000 automated DNA sequencer (LI-COR, Inc., Lincoln, NE) (21). The electrophoresis buffer was composed of 89 mM Tris, 89 mM Boric acid, 2 mM EDTA, pH 8.0 (1X TBE). Gels were 25 or 33 cm in length, 0.25 mm in thickness, and composed of either 7% (25 cm gels) or 6% (33 cm gels) Long Ranger (AT Biochem; Malvern, PA) containing 7 M urea. In addition, 25 cm gels contained 0.4X TBE while 33 cm gels contained 1.2X TBE. The 25 cm gels were run with 0.4X TBE running buffer at 2000 volts constant, while 33 cm gels were run with 1.0X TBE running buffer at 1500 volts constant. Running temperature was maintained at 50°C by a heated thermostatic plate in contact with the back plate of the gel. Square toothed combs for 32 or 64 wells were used during gel casting. Samples were denatured at 95°C for 2–3 minutes then quickly cooled on ice. Diluted samples (1.5 μL volumes) were loaded onto a gel mounted in a LI-COR Model 4000 automated DNA sequencer and electrophoresed. For most of the results shown in this research a sizing ladder consisting of one lane of a standard sequencing reaction was used. The raw data depicting the STR alleles is displayed as an autoradiogramlike image on the computer screen and stored as a file on the hard drive of the computer.

Results and Discussion

STR analysis is becoming an integral part of forensic DNA analysis. The aim of this research was to detect STR alleles from dried bloodstains with minimal DNA extraction and to automate the analysis. The authors also wished to detect STR alleles using Chelex extracted DNA from bloodstains, saliva, hair roots, semen, vaginal swabs and other types of evidence most commonly encountered in forensic cases. Since Taq polymerase is primarily used in PCR amplification of forensic samples, multiplexing using this enzyme was also attempted so that at least three loci could be detected in a single reaction.

Using Tth polymerase and hot start PCR it was possible to directly amplify and detect STR alleles from bloodstains on 100% cotton or cotton-polyester fabric and dried flakes of blood. All five loci amplified and STR alleles were detected using only a high temperature incubation for DNA extraction. Figure 3 exhibits

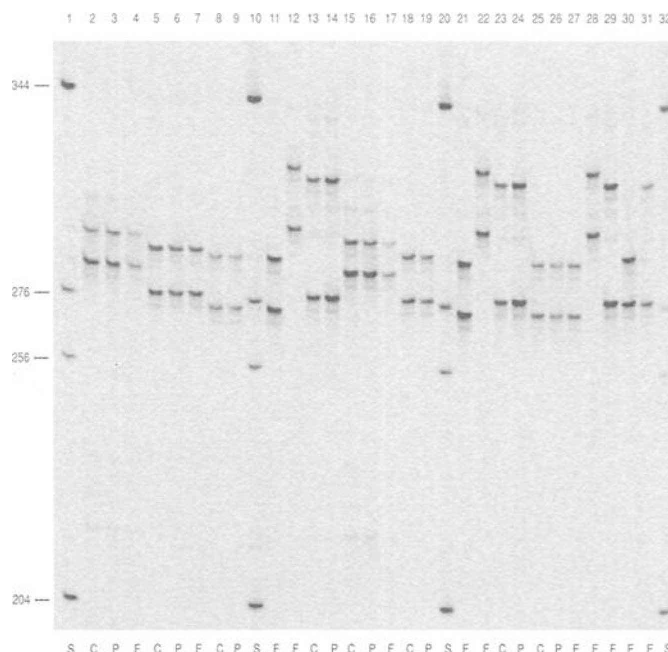

FIG. 3—*Allelic profiles from bloodstains for the ACTBP2 locus using a 25 cm gel. Lanes 2–4, 5–7, 15–17 and 25–27 represent samples from four individuals. In each set three types of bloodstains (100% cotton fabric (C), cotton-polyester fabric (P) and dried flakes of blood (F), respectively) are represented. Lanes 8–9, 13–14, 18–19 and 23–24 are from bloodstains using 100% cotton and cotton-polyester fabrics, respectively. Lanes 11, 12, 21, 22 and 27–31 are from dried flakes of blood. Lanes 1, 10, 20 and 32 are molecular weight size standards (S) shown to the left of the image (in base pairs).*

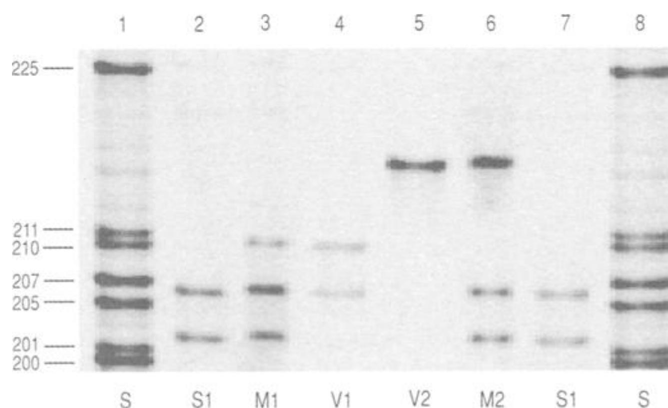

FIG. 4—*Allelic profiles for the HUMTHO1 locus from individual and mixed bloodstains on a 25 cm gel. In this simulated case lanes 2 and 7 represent bloodstains from a suspect (S1) amplified from 100% cotton and cotton-polyester fabric, respectively. Lanes 4 and 5 represent bloodstains collected on 100% cotton from two victims (V1 and V2, respectively). Lane 3 (M1) represents a mixture of blood from S1 (bloodstain 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).*

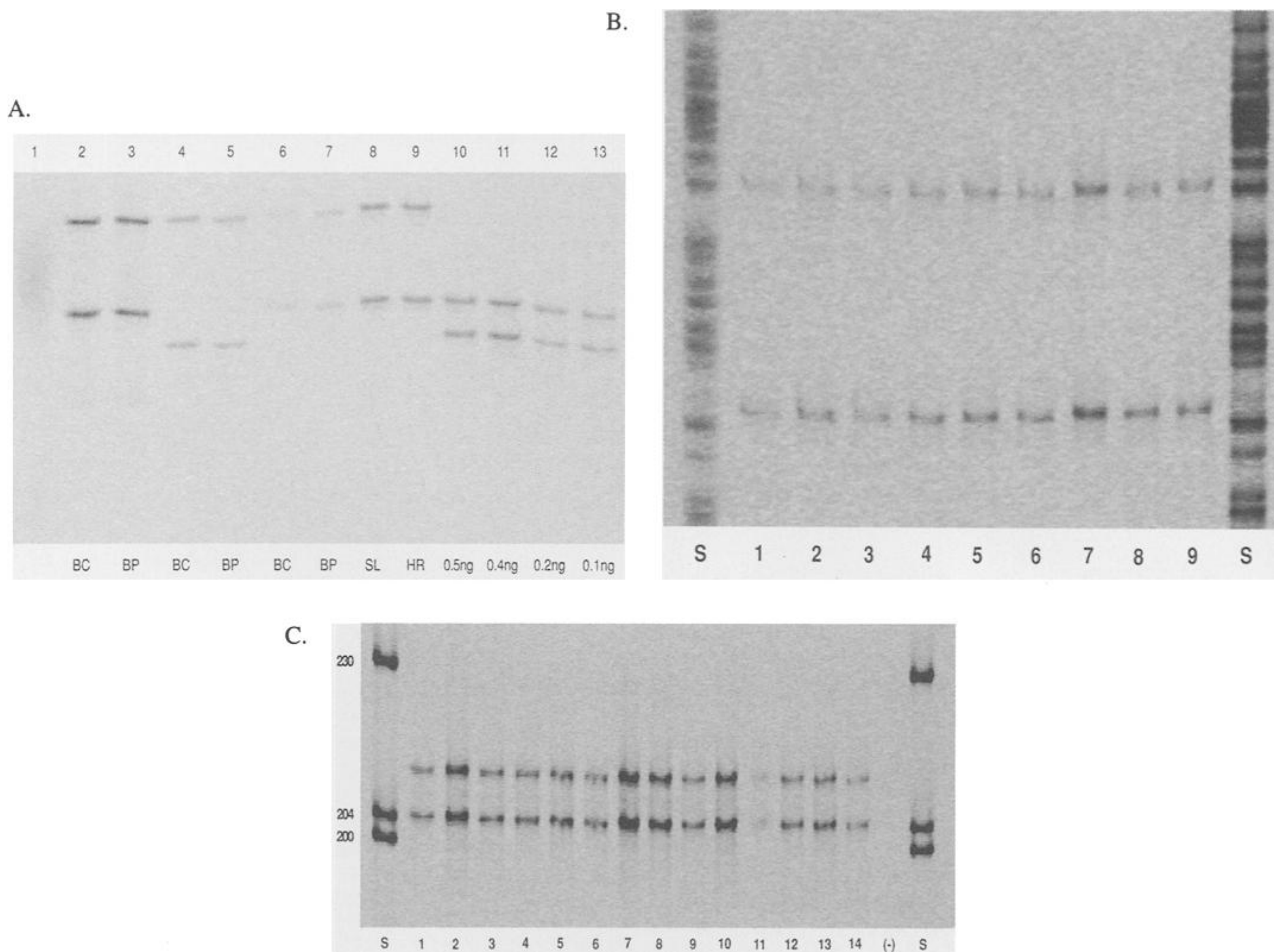

FIG. 5—(A) *Allelic profiles of various individuals for the HUMTHO1 locus from blood, saliva and hair using a 33 cm gel. Lane 1 is a negative control lacking template DNA in the amplification mixture. Lanes 2, 4 and 6 are bloodstains from 100% cotton fabric (BC) amplified directly with high temperature DNA extraction. Lanes 3, 5 and 7 are bloodstains from which DNA was extracted by the Chelex procedure from cotton-polyester (BP) fabric. STR profiles of lanes 7–9 are from DNA extracted from bloodstain, saliva (SL) and hair (HR), respectively, from a single donor. A control DNA sample of known quantity (listed below each lane) was used as template in lanes 10–13. The portion of the gel shown does not contain a molecular weight size marker.* (B) *Allelic profiles for the ACTBP2 locus using Chelex extracted DNA from simulated forensic samples from a male volunteer. Lane 1—toothbrush; lane 2—perspiration; lane 3—saliva; lane 4—cigarette butt; lane 5—toenail; lane 6—hair; lane 7—blood; lane 8—semen (cloth); lane 9—semen (paper); S—molecular weight standard.* (C) *Allelic profiles for the HUMTHO1 locus using Chelex extracted DNA from simulated forensic samples from a female volunteer. Lane 1—vaginal swab; lane 2—vaginal wipe; lane 3—blood; lane 4—saliva; lane 5—toenail; lane 6—hair; lane 7—earring; lane 8—perspiration; lane 9—toothbrush; lane 10—cigarette butt; lane 11—envelope flap; lane 12—skin wipe; lane 13—menstrual blood; lane 14—hair; lane 15—negative control; S—molecular weight standard.*

STR patterns for the ACTBP2 locus from the three types of bloodstains collected from various individuals. The 37 cycle amplification protocol produces a very intense signal with high quality DNA but is necessary for the amplification of DNA, which might be of poor quality as is sometimes encountered in forensic samples. A two-stage thermal cycling protocol was used. Since the template consists of high complexity human genomic DNA, the first stage used a higher temperature (67°C) annealing step to increase the specificity of amplification. Once the correct PCR product increases in concentration, the annealing temperature is lowered (62°C) to allow increased yield of the amplification product. This two-stage procedure may help prevent nonspecific amplification which may be a concern when larger cycle numbers (37X) are used. Although the protocol requires addition of a second mixture after the high temperature incubation is completed, optimal results were obtained using the procedure as described. Addition of the second mixture subsequent to high temperature DNA extraction decreases thermal degradation of deoxynucleotides, IR-labeled primers and polymerase.

Using the described protocol it was also possible to perform mixed stain analysis using blood from two individuals. In these cases a 1 mm thread of bloodstain from each of the two individuals was amplified in a single reaction tube. As illustrated in Fig. 4, the allelic profiles of two individuals were amplified in a single reaction mixture with minimal DNA extraction. It is also possible to amplify and detect Chelex extracted DNA from body fluid mixtures (such as, blood and saliva) using either Tth or Taq polymerase in a single reaction (data not shown). This is especially useful in sexual assault cases where a victim's body fluids are often mixed with those of the assailant.

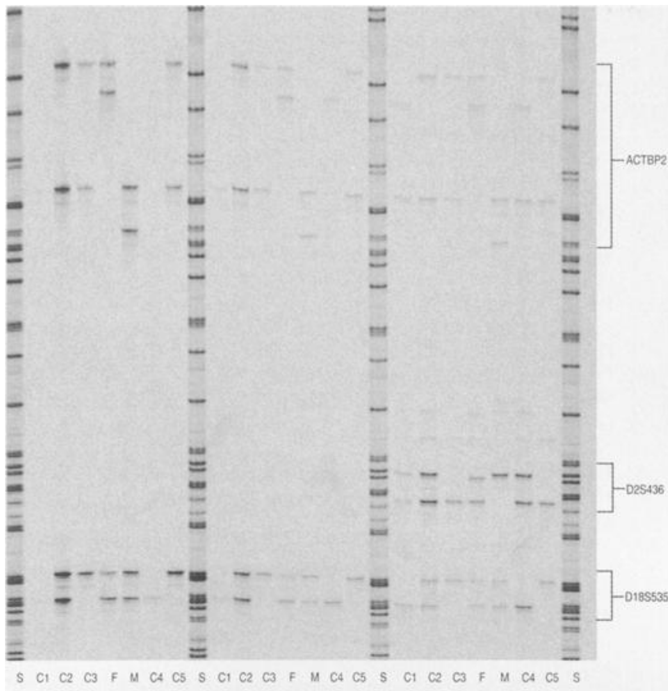

FIG. 6—*Allelic profiles of a nuclear family on a 33 cm gel. F = Father, M = Mother, C1 through C5 = Children, S = Molecular weight size standard consisting of one lane of a standard sequencing reaction. STR Loci are designated on the right side of the image. The left and center reaction sets contain ACTBP2 and D18S535 amplification primers, while the right set contains ACTBP2, D2S436 and D18S535 primers.*

Results from direct amplification of bloodstains were validated by comparison to amplified Chelex extracted DNA obtained from bloodstains, buccal swabs, and hair roots (Fig. 5*A*). The same STR band pattern is obtained with both extraction protocols. Various other types of simulated forensic samples were also analyzed. Figure 5*B* demonstrates allelic profiles for the ACTBP2 locus from Chelex extracted bloodstain, saliva, hair, semen, etc. collected from a male volunteer. Figure 5*C* shows a similar analysis using the HUMTHO1 locus performed with samples from a female volunteer. All analyses show identical allelic profiles for each individual for a wide variety of different sample types. The ability to perform STR pattern analysis on semen and vaginal samples is particularly useful since sexual assaults comprise the majority of the cases requiring DNA analysis.

For direct detection of STR loci from bloodstains Tth polymerase appears to be more tolerant of inhibitors present in blood than Taq polymerase, which failed to amplify STR loci directly from bloodstains. Tth polymerase also has the advantage of being more heat tolerant during high temperature incubation than Taq, according to the manufacturer (22). However, Taq polymerase amplified all of the alleles when DNA was Chelex extracted from bloodstains. For STR typing of saliva and hair samples using Tth polymerase it was also necessary to Chelex extract DNA. It might be expected that saliva could also be directly typed as bloodstains were, however this was found not to be the case. We hypothesize that the desiccation occurring during drying of bloodstains promotes release of DNA from the cells. Buccal cells from saliva were added directly to the amplification reaction and thus did not experience this. STR alleles have also been detected from Chelex extracted DNA from saliva and hair roots using Taq polymerase (data not shown).

Multiplex reactions using two or three sets of primers in a single reaction tube were performed using Taq polymerase (Fig. 6). As the example of the family study shows, this procedure can be used for paternity analysis as well. Throughput can be increased by using a 64-well comb for gel casting. By loading a 64-well gel twice and multiplexing with three primer pairs, it is possible to type three loci from 120 individuals in a day. We have obtained somewhat poorer results (that is, lower STR band intensities) with the multiplexing procedure. This seems to be indigenous to the use of multiple primer sets since amplifications using Chelex extracted template and one primer set yield good quality results. Additional optimization of this multiplex amplification protocol may allow these loci to be utilized in a standardized triplex analysis.

This study uses automated infrared fluorescence technology (21) as a means of detecting STR polymorphisms. The near infrared region of the spectrum provides distinct advantages over more commonly-used ultraviolet and visible wavelengths. Infrared absorbance and fluorescence emission of solvents and biomolecules is minimal (7). The extremely low background and high sensitivity allow detection of diminutive quantities of labeled molecules. Using this amplification and detection methodology we obtain excellent results from 0.1 nanogram of template DNA (Fig. 5*A*) and have been able to detect STR bands down to approximately 15 picograms of high complexity human genomic DNA template using both the Tth and Taq protocols (data not shown). Actual raw data is collected on-line in real time, visualized during gel electrophoresis and can be stored automatically into a database. A 25 cm denaturing gel run at 2000 V (constant) is used for rapid resolution and visualization of STR bands. The separation distance of 15 cm allows migration of the DNA fragments to the scanning detector in less than 30 min. For better separation of allelic bands a 33 cm or longer gel can be used. The total time for direct detection of STR alleles from bloodstains including PCR amplification is less than four hours using a 25 cm gel. The same gel can be reloaded and run up to three times to increase throughput.

Close examination of the images in Figs. 3 through 6 reveals the presence of several types of artifactual bands. The STR bands in Fig. 3 produced from the ACTBP2 locus tend to have a fainter band approximately 4 bases below them. This is thought to be caused by strand slippage along the tetrameric repeat region resulting in amplification products that are four bases shorter. They are more pronounced in the smaller alleles possibly due to PCR amplification being more efficient for shorter targets. These bands are also visible in multiplex reactions (Fig. 6) at the ACTBP2 locus, which seems to have more tendency to produce strand slippage than other loci. Also present in Fig. 3 are faint bands occurring approximately 20 basepairs above the authentic STR band. Our experience indicates that this artifact is associated with the use of the M13 tail attached to one STR primer, although the exact mechanism producing these spurious bands is not presently known. This artifact is also clearly seen in Fig. 6 at the D2S436 locus, which in our hands had a much greater propensity to produce all types of artifacts than any other loci used. Preliminary results indicate that use of IR-labeled dATP to internally label STR products amplification strategy no. 3) eliminates this artifact. The D2S436 and D18S535 loci shown in Fig. 6 have a faint band one base under the primary band. These artifacts (n-1 bands) are not uncommon among PCR amplification products and their origin remains to be clarified. Smaller sample quantities loaded onto the gel reduces the visual impact of these various artifacts while ongoing work attempts to minimize and/or eliminate them.

This study demonstrates that STR locus patterns can be generated from a wide variety of forensic-like samples and detected using a high sensitivity IR fluorescence scanning system. This data can aid in identification of individuals in forensic casework. A variety of molecular weight markers have been used in these images, however none of these are ideal for forensic identification. An allelic ladder composed of an STR band for every naturally occurring repeat number is an optimal standard for sizing and individualizing each allele under analysis (23), and we are currently assembling such a ladder. When such a ladder becomes available compilation of population databases can be rapidly accomplished. STR band pattern data can be conveniently managed using image analysis software such as RFLPscan from Scanalytics (Billerica, MA). With appropriate placement of standards on the gel the software can compensate for electrophoresis anomalies (such as "smiling") and can accurately size the alleles by number of basepairs or repeat units. Such methodology will ultimately allow accurate high throughput STR analysis for forensic as well as other applications such as gene mapping, paternity testing and diagnostics.

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