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Identification of Male Bloodstains by Dot Hybridization of Human Y Chromosome-Specific Deoxyribonucleic Acid (DNA) Probe

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ABSTRACT: The sex determination of bloodstains was performed using a human Y chromosome-specific (DNA) fragment of 1.9-kb length as a hybridization probe. The DNA samples were taken from 1- and 4-week-old bloodstains of males and females, respectively. Strong signals with male DNA were observed by Y-probe, while faint signals with female DNA were detected. In addition, clear signals were observed in the extract samples from male bloodstains (16-week-old) on paper. Dot hybridization of the Y-probe would be widely applicable to studies on sex determination of medicolegal materials such as blood, bloodstains, teeth, and cadaverous parts.

KEYWORDS: forensic science, human identification, blood, deoxyribonucleic acid (DNA), dot hybridization, bloodstains, Y chromosome-specific DNA probe

Since it has been demonstrated that fluorescent substance binds selectively with a certain portion of the chromosome, this method has been applied to the sex determination of human beings [*1,2*]. Although this fluorescence method is quite useful for the sex determination of bloodstains and teeth in forensic science practice [*3,4*], it must be done by an experienced investigator.

In general, ribonucleic acid-deoxyribonucleic acid (RNA-DNA) and DNA-DNA hybridization reactions are the basis of many assays in recombinant DNA technology. Two single-stranded molecules anneal to form a base-paired duplex in hybridization reactions. These reactions techniques are now extensively used for clinical research. Moreover, DNA probes are being used in the analysis of human DNA for the diagnosis of hereditary disorders and also infections. Fetal sex determination by DNA hybridization of Y chromosome-specific probe is of great value in the prenatal diagnosis of X-linked recessive conditions [*5–7*]. For the purpose of sex determination of bloodstains, we have isolated DNA samples from male and female bloodstains by the two different methods.

In this paper, we describe a new method for sex determination of bloodstains by hybridizing DNA from bloodstains with a Y chromosome-specific DNA probe.

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Materials and Methods

Isolation of DNA from Male and Female Bloodstains

Blood samples were obtained from ten individuals (five males and five females). Whole blood was dropped into a clean 500-mL glass beaker (1 mL of blood) or onto clean paper (0.5 mL of blood), and then dried by a hair dryer, followed by storage at room temperature (one, four, and sixteen-week-old samples). The size of the bloodstain on paper was approximately 5.5 by 5.5 cm.

Fresh blood (2 mL) or bloodstains in glass beakers were suspended in blood lysis buffer [*8*] containing 0.32*M* sucrose, 5m*M* magnesium chloride ($MgCl_2$), 1% Triton X-100, and 10m*M* Tris-hydrogen chloride (HCl) (pH 7.6), respectively. After centrifugation at 2000x*g* for 15 min at 4°C, the pellets were suspended in DNA lysis buffer containing 1% sodium dodecyl sulfate (SDS), 24m*M* ethylenediaminetetraacetate (EDTA), 75m*M* sodium chloride (NaCl), and 10m*M* Tris-HCl (pH 8.0). A glass rod may be used to aid this process and to disperse clumps of material. Proteinase K was added to final concentrations of 150 μg/mL. After incubation at 37°C overnight or at 50°C for 30 min, solid cesium chloride (CsCl) was added to the final 50% concentration and dissolved by gently inverting. Ethidium bromide (100-μL solution containing 1 mg of ethidium bromide/about 2 mL of DNA sample solution) was added and mixed with the samples. The tubes containing the sample were sealed and loaded into a rotor, which was centrifuged at 20°C overnight in a Hitachi 55P-72 ultracentrifuge [*9*]. DNA bands were removed by side puncture of the tube with the needle. Ethidium bromide was removed by extraction with isoamyl alcohol saturated with water and CsCl about four to five times. After dilution with two volumes of water, sodium acetate was added to a final concentration of 0.3*M* and then the DNA was precipitated with two volumes of chilled absolute ethanol at −20°C. After again washing with chilled 80% ethanol and centrifugation, DNA samples were redissolved in 10m*M* Tris-HCl (pH 7.5) buffer containing 1m*M* EDTA.

Optical absorbance can be used as an accurate measure of DNA concentration. An approximation of this absorbance is that double-stranded DNA at 50 μg/mL in aqueous solution has an A^{1cm}_{260nm} of 1.

Labeling of DNA

The probe was labelled with deoxycytidine 5′-[α-^{32}p] triphosphate (Amersham) by the multiprime labelling kit (Amersham). Y chromosome-specific DNA probe was propagated as cloned inserts in the phage (cloned from human Y chromosome-specific gene library λ-Charon 21A constructed by Dr. Marvin A. Van Dilla, Lawrence Livermore National Laboratory, CA). The cloned phage Charon 21A contains 1.9-kb Hae III insert.[3]

Dot Hybridization

The isolated DNA samples from fresh blood or bloodstains were heated to 100°C for 5 min and chilled on ice. Each sample was spotted on nylon membrane (Amersham). As for the 16-week-old bloodstains on the paper, a 1-mm^2 piece of paper with dried bloodstains was soaked in 10 μL of 10m*M* Tris-HCl (pH 7.5) containing 1m*M* EDTA and 1% SDS for 30 min at 37°C. The clear extracts were spotted directly. These membranes were soaked in denaturing solution (1.5*M* NaCl, 0.5*M* sodium hydroxide [NaOH]) and neutralizing solution (1.5*M* NaCl, 0.5*M* Tris-HCl pH 7.2, 1m*M* EDTA) for 1 min and then dried. For fixing DNA blots to nylon membrane, an ultraviolet (UV) lamp (305 nm) was used for 2 to 3 min, held about 15 cm from the membrane. The membrane was prehybridized for 1 h at 65°C in a solution

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containing 6 × SSC (six times the concentration of 1 × SSC) (1 × SSC = 0.15*M* sodium chloride, 0.015*M* sodium citrate), 0.5% SDS, ×5 Denhardt's solution [*10*], and 0.1 mg/mL of salmon sperm DNA. For hybridization, the DNA probe was added to the prehybridization solution. Hybridization with the Y Chromosome-specific DNA probe was performed for 5 to 10 h at 65°C, followed by washing in (1) 2 × SSC for 15 min twice, (2) 2 × SSC containing 0.1% SDS for 30 min, and (3) 0.1 × SSC for 10 min at 65°C, respectively. Autoradiographs were exposed at −80°C for 1 to 3 h with an intensifying screen.

Densitometry of autoradiographs was carried out using 570 nm.

Results and Discussion

DNA analysis is widely used for genomic disorders. A recombinant DNA probe was a suitable marker for antenatal diagnosis of sex-linked disorders such as Duchenne muscular dystrophy, hemophilia and Lesch-Nyhan syndrome. The present study on sex determination using the Y chromosome DNA probe was undertaken because of the importance of bloodstain analysis in the forensic sciences. DNA samples were isolated from male and female bloods and bloodstains by two different methods such as centrifugation and 1% SDS extraction. From four-week-old bloodstains (1 mL) 15 to 25 μg of DNA were recovered in a glass beaker. Figure 1 shows the approximate size of the genomic DNAs from male and female bloodstains identified by ethidium bromide staining of agarose gel. No band smaller than 23 kb was observed in DNAs from one- and four-week-old bloodstains.

Although the preliminary communications were reported [*11,12*], our new recombinant DNA used here demonstrated a high specificity for male DNA and hybridized to EcoR I-digested male DNA but not to female DNA in Southern blotting analysis.[3] Dot hybridizations were carried out to determine the sex of blood (Fig. 2) and bloodstains (Fig. 3) using the Y-chromosome probe. Apparently, strong signals with male DNA were observed by the

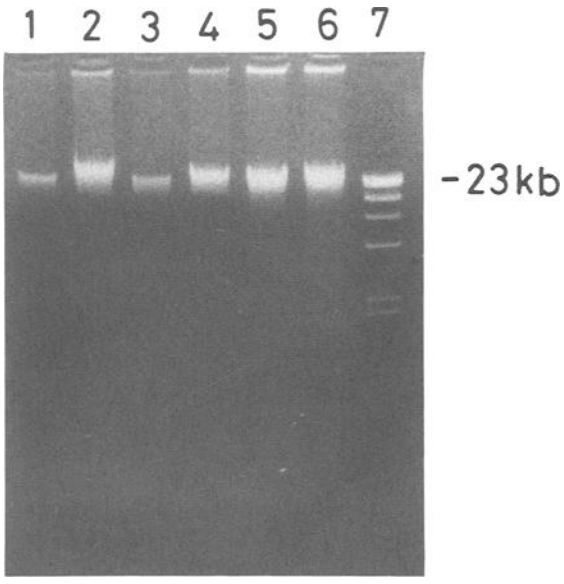

FIG. 1—*Analytical gel electrophoresis (0.8% agarose) of DNA samples (0.5 to 0.8 ug) isolated from male and female bloodstains.* 1 = *male fresh blood,* 2 = *female fresh blood,* 3 = *male 1-week-old bloodstains,* 4 = *female 1-week-old bloodstains,* 5 = *male 4-week-old bloodstains,* 6 = *female 4-week-old bloodstains, and* 7 = *Hind III digested* λ *phage DNA.*

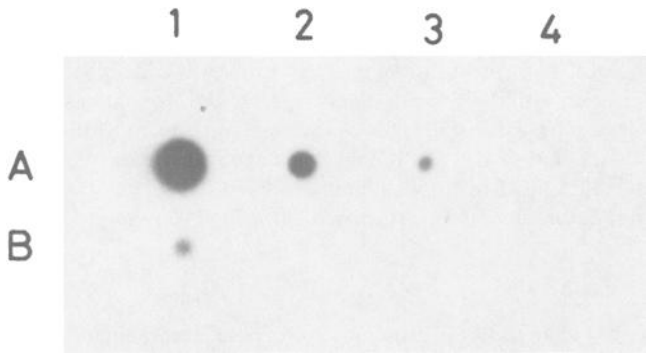

FIG. 2—*Dot hybridization for sex determination of fresh bloods. DNAs were isolated from fresh blood as described under Materials and Methods.* A = *DNA from male blood,* B = *DNA from female blood,* 1 = *100 ng of DNA,* 2 = *50 ng,* 3 = *12.5 ng, and* 4 = *3 ng.*

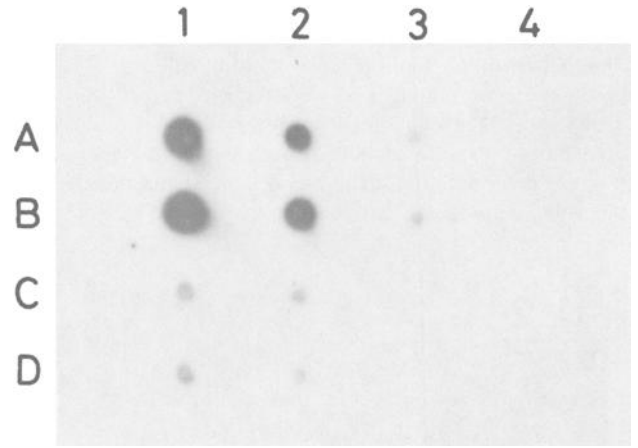

FIG. 3—*Dot hybridization for sex determination of bloodstains. DNAs were isolated from the bloodstains as described under Materials and Methods.* A = *DNA from 1-week-old bloodstain of male,* B = *DNA from 4-week-old bloodstain of male,* C = *DNA from 1-week-old bloodstain of female,* D = *DNA from 4-week-old bloodstain of female,* 1 = *90 ng of DNA,* 2 = *45 ng,* 3 = *10 ng, and* 4 = *2.5 ng.*

Y-probe, while faint signals with female DNA were detected. Figure 4 shows the corresponding traces obtained by scanning the autoradiographs. Compared with the female bloodstains, approximately 15-fold specificity of the probe was demonstrated in the male bloodstains. In addition, note that the same results were obtained from extract samples of 16-week-old bloodstains on paper (Figs. 5 and 6). This indicates that the extraction step with phenol/chloroform (1:1) was not required to isolate DNA from bloodstains on paper.

It is essential for forensic scientists to induce information as much as possible from bloodstains at the scene or on the clothes of suspected persons. A number of analytical procedures for blood identification have been established. Up to now, immunologic and biochemical analysis of bloodstains has been frequently used [*13,14*]. However, detectability of antigens

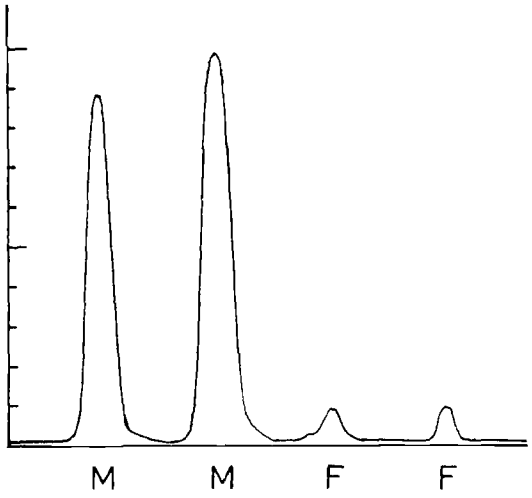

FIG. 4—*Densitometer scan of male and female bloodstains dot hybridization from left-hand side (1 = A, B, C, and D) of Fig. 3.*

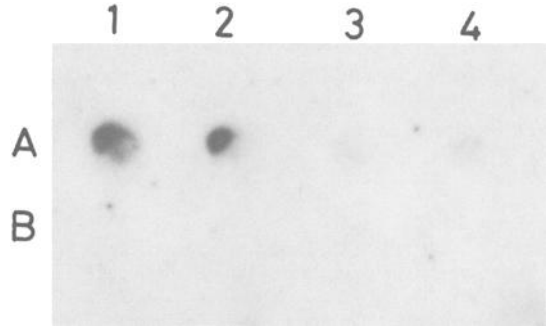

FIG. 5—*Dot hybridization for sex determination of bloodstains. The extractions from a 1-mm² piece of paper with a dried bloodstain were directly spotted on nylon membrane.* A = *extract of 16-week-old bloodstains of male,* B = *extract of 16-week-old bloodstains of female,* 1 = *2.5-uL extract,* 2 = *1.5-uL extract,* 3 = *0.5-uL extract, and* 4 = *0.2-uL extract.*

and enzymes in dried blood ranges from a few days to years, depending on the conditions of specimen exposure [*15*].

It is very important to identify the sex of the person from whom a bloodstain originated. Therefore, the hormonal radioimmunoassay [*16,17*] as well as the analytical method of Y chromosome has been used for sex determination of bloodstains. Recently, two papers [*18,19*] reported that the Y-probe could be used in the sex determination of bloodstains using dot hybridization. However, compared with these methods, our paper described a more rapid (one to two days) dot hybridization procedure. To achieve still greater discrimination of bloodstains, DNA analysis should be carried out in the forensic science arena. DNA hybridization by the Y chromosome specific-probe has become a powerful tool to determine the sex of bloodstains.

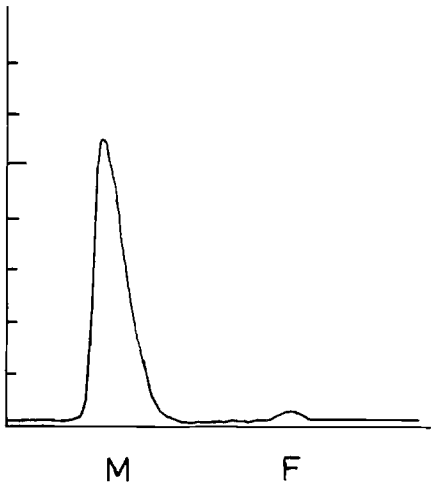

FIG. 6—*Densitometer scan of male and female bloodstains dot hybridization from left-hand side (1 = A and B) of Fig. 5.*

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