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Sex Determination of Bloodstains by the Use of a Ribonucleic Acid (RNA) Probe

REFERENCE: Kobayashi, R. and Matsuzawa, S., **"Sex Determination of Bloodstains by the Use of a Ribonucleic Acid (RNA) Probe,"** *Journal of Forensic Sciences.* JFSCA, Vol. 34, No. 5, Sept. 1989, pp. 1078-1081.

ABSTRACT: An asymmetric ribonucleic acid (RNA) probe, which represents a Y-chromosome specific nucleotide sequence, was applied to the sex origin of bloodstains by using a nonisotopic Southern hybridization method. The RNA probe was in vitro transcribed from the recombinant of the Y-chromosome specific deoxyribonucleic acid (DNA) sequence and transcription-vector Bluescript SK M13$^+$. The use of this RNA probe can lower the detection limit of the nonisotopic DNA analyzing method to 1/8 of that reached by our previously reported method in which a DNA probe was used.

KEYWORDS: forensic science, ribonucleic acid, blood, human identification

We previously reported a nonisotopic deoxyribonucleic acid (DNA) analyzing method for sex identification from fresh blood and dried bloodstains [*1*]. The study was performed with a human Y-chromosome specific repeated DNA (3.5-kilobase [kb] pair EcoRI fragment) and a recombinant plasmid containing this sequence. Although the use of these DNA probes results in a considerable degree of sensitivity, recent developments in recombinant DNA methods should allow us to expect an increase in sensitivity with the use of ribonucleic acid (RNA) probes [*2-5*].

In this study, the sensitivity and the usefulness of a biotin-labeled RNA probe in a nonisotopic Southern hybridization method for sex determination is reported.

Materials and Methods

Synthesis of Asymmetric RNA Probe

The human Y-chromosome specific DNA, a 3.564 kb pair EcoRI fragment (pHY10), was kindly supplied by Dr. Y. Nakahori, Department of Congenital Abnormalities Research, National Children's Medical Center [*6*]. Bluescript SK M13$^+$ cloning vector (BS-SK+) was purchased from Stratagene Cloning Systems Co. (La Jolla, California).

A biotin-labeled RNA probe was in vitro synthesized. The procedures described by Maniatis et al. [*7*] were followed for (1) subcloning DNA fragments of pHY10 into BS-SK+, (2) the transformation into *E. coli* XL1-Blue (Stratagene Cloning System Co.), (3) the screening of recombinants, (4) the propagation of recombinants, and (5) the subsequent preparation

Received for publication 3 Aug. 1988; revised manuscript received 19 Dec. 1988; accepted for publication 20 Dec. 1988.

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of plasmids from the recombinants. The plasmid was linearized with BamHI (Takara Shuzo Co., Kyoto) and then phenol-extracted and ethanol-precipitated. Transcription of the linearized plasmid DNA with T7 RNA polymerase was done according to the manufacturer's instructions.

The following were mixed in order at room temperature: 10 μL of diethylpyrocarbonate (DEPC)-treated water; 2 μL of mix (400m*M* Tris-hydrochloric acid [HCl]; pH 8.0/200m*M* magnesium chloride [$MgCl_2$]/50m*M* dithiothreitol [DTT]); 1.5 μL of mix (6.7m*M* adenosine 5′-triphosphate [ATP]/6.7m*M* cytidine 5′-triphosphate [CTP]/6.7m*M* guanosine 5′-triphosphate [GTP] [Pharmacia, Uppsala]); 2 μL of 10m*M* biotin-11-uridine triphosphate (UTP) (BRL, Gaithersburg, Maryland); 1 μL of human placental ribonuclease inhibitor at 25 U/μL (Amersham, Buckinghamshire); 1 μL of 0.2*M* DTT; 2 μL of the restricted DNA template at 1 μg/μL in water; and 0.5 μL of T7 RNA polymerase at 50 U/μL (BRL). After storage for 1 h at 40°C, 2 μL of tRNA at 20 mg/mL (Boehringer, Mannheim), 2 μL of 3*M* sodium acetic acid (CH_3CO_2Na), and 50 μL of ethanol were added, and the reaction mixture was incubated for 30 min at −80°C. After centrifugation and removal of supernatant, precipitates were dried and resuspended to 100 μL of DEPC-treated water. A DNA probe, as prepared in the previous study [*1*], was used as a control.

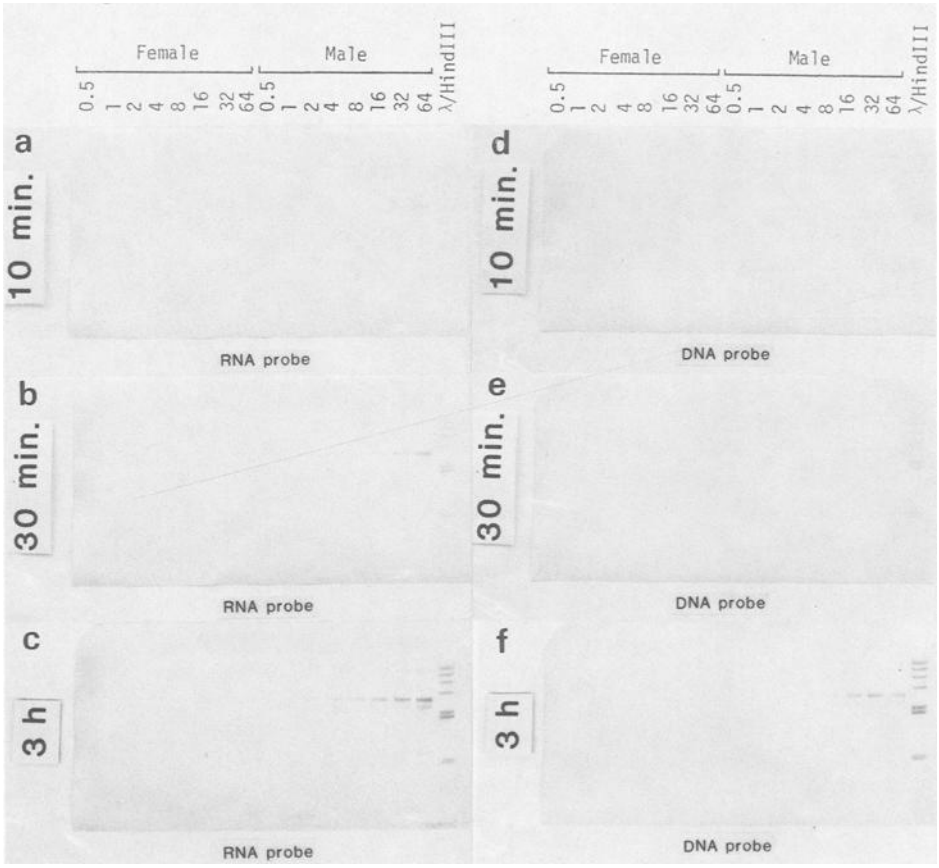

FIG. 1—*Comparison of two Southern hybridization patterns with the RNA probe* (a, b, c) *and the DNA probe* (d, e, f). *Change in color was photographed at 10 min, 30 min, and 3 h after starting the staining procedure. Numbers at top indicate the amounts in nanograms of EcoRI-treated DNA. Lane λ/HindIII is for molecular weight standards.*

Extraction of DNA and Southern Hybridization Method

The procedures for extraction of DNA and performing Southern hybridization were followed as in our previous report [*1*]. Bloodstains used were kindly supplied from the Metropolitan Police Department (Tokyo). Specimens were not used if the sexes of murdered or wounded persons were in doubt. Stained fabrics (about 1 cm^2) were cut into small pieces before extraction.

Results and Discussion

Figure 1 shows two hybridization patterns of DNA specimens from two blood samples—one male and one female. Patterns obtained with a biotin-labeled RNA probe and those with a biotin-labeled DNA probe are compared. Although the patterns are essentially the same, the appearance time of the male specific 3.4-kb band was apparently shorter in testing with the RNA probe than with the DNA probe. For the detection of 20- to 30-ng/lane male DNA, the 3.4-kb band appeared after the staining period of 10 min, whereas it required about 30 min to reach a similar intensity of staining with the DNA probe. The detection limit of the

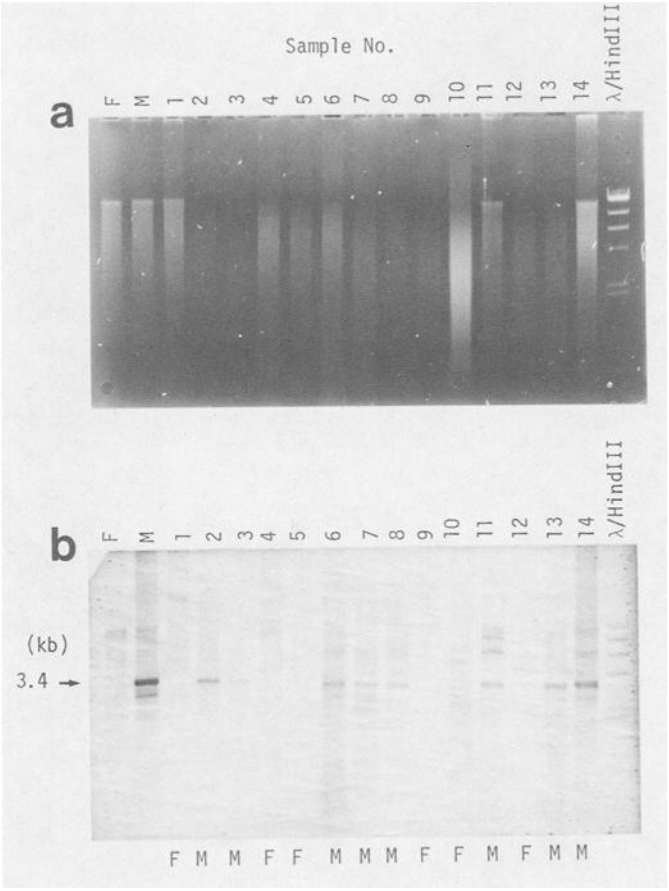

FIG. 2—*Sex determination from bloodstains, collected from crime scenes, by a nonisotopic Southern hybridization method using an RNA probe:* (a) *fluorescences of ethidium bromide added gel and* (b) *hybridization patterns of EcoRI-treated DNA.* M = *male and* F = *female.*

3.4-kb band was about 1 ng/lane with the RNA probe (Fig. 1*c*), which is about eight times lower than that obtained with the DNA probe (Fig. 1*f*).

A series of sex identification experiments with bloodstain specimens, picked up at crime scenes, was performed by using the RNA probe. As in the patterns showing the fluorescence of ethidium bromide added gel (Fig. 2*a*), each electrophoresed lane was charged with sufficient quantities of DNA. After staining, the patterns as shown in Fig. 2*b* were obtained. The 3.4-kb band appeared on the patterns of all male specimens. Femaleness of the specimens was also determined according to the criteria described in our previous report [*1*]. Of 45 specimens (26 males and 19 females), all of the sexes were accurately determined. No false positive band appeared at the position of 3.4 kb. Further studies with forensic science practical cases are to be reported elsewhere.

Conclusion

These above results indicate the superiority of the RNA probe over the DNA probe. Because the RNA probe has a high hybridization efficiency, sensitivity of the test is increased and the period of time necessary for obtaining the resulting stains is shortened. Preparation of the RNA probe is not difficult, although it requires a subcloning procedure, which is unnecessary in the preparation of the DNA probe.

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