Rapid Sex Determination Using Restriction Enzymes Digestion

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In criminal investigation, the rapid determination of sex origin of bloodstains found at crime scenes or on clothing evidence provides an important clue to the identification of the suspect. Up to now, sex determination has been performed with morphological methods such as visualization of sex chromatin material (Zech 1969) or sex hormone analysis (Brown 1981 ; Yamamoto et al 1984).

Recently, several techniques dealing with molecular biology have been proposed by analyzing the Southern hybridization pattern of DNA to detect the presence of a Y chromosome specific DNA fragment (Vergnaud et al 1984; Fukushima et al 1988; Kobayashi et al 1988).
We report here a rapid method of sex identification using only a restriction enzyme digestion.

MATERIALS AND METHODS

The material consisted of :
- 6 vaginal swabs
- 6 bloodstains on coton and nylon fabric (150 µl)
-12 whole blood samples for paternity testing (0.5 ml).

DNA Isolation

Stains and swabs were cut into small pieces and incubated with proteinase K (150 µg/ml) and sodium dodecylsulfate (1 %) for two hours at 37°C.
Whole blood samples were treated with a lysis buffer (0.35 M sucrose, 10 mM Tris HCl pH 7.6, 1 % Triton X 100) before similar incubation with proteinase K.

DNA Extraction

DNA extraction was performed with phenol, chloroforme and isoamyl alcool following by dialysis against TE buffer for 2 x 4 hours.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

Yield Gel

High Molecular Weight (HMW) DNA was estimated on a yield gel (0,9 % agarose, 2 hours at 80 volts) after staining with ethidium bromide ; 2 µg of HMW DNA being required to get successful result.

Digestion with Dde 1 or HAEIII

Samples of 2 µg of DNA were digested with 80 IU HAE III (Boehringer Ingelheim) or 80 IU Dde1(Boehringer Ingelheim) during 2 x 2 hours at 37°C.

Then DNA fragmentation was visualized by ethidium bromide staining after electrophoresis on 1.1 % agarose gel for 15-20 hours at 20 volts.

RESULTS AND DISCUSSION

Enough HMW DNA was extracted from 2 vaginal swabs, 5 blood stains and from all blood samples.
A 2.3 kb band was found to appear on the patterns of male DNA after digestion with HAE III. A similar band at 3.0 kb was identified with Dde 1. Confirmation was obtained by hybridization of X and Y probes on a nylon membrane (M.S.I.) after Southern transfer.
Appearance of the 2.3 kb or 3.0 kb bands among male patterns according to the selected restriction enzyme has been shown to be related to a human specific Y chromosome repeated sequence (Cooke et al 1982; Fowler et al 1987).
Thus male and female specimens can be clearly discriminated.
The method has been found to be sensitive enough to be successfully applied in forensic cases since no more than 2 µg of extracted DNA are necessary to perform the test.
In addition, this nonisotopic method does not require the use of a specifically designed laboratory or an expensive equipement.
Thus, it may be useful in forensic practice as for checking the presence of DNA of male origine in vaginal swabs in case of rape, in bloodstains in criminalistics or in blood samples in paternity testing.

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