Analysis of Case Work Samples by Single Locus VNTR Polymorphisms

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INTRODUCTION

For the past several years, restriction fragment length polymorphism (RFLP) analysis of DNA isolated from forensic samples has been used to provide evidence in forensic cases. To date, Lifecodes Corporation has analyzed over 2000 cases and has presented testimony in over 100 US court cases in 21 states. Examples of casework samples are presented.

EXTRACTION

DNA from semen stains and vaginal swabs is obtained by a differential lysis procedure (Giusti et al. 1986) to enrich for male DNA and the supernatant (female DNA) can be used to establish the victim's DNA pattern. Liquid blood and dried blood stains are processed for DNA as previously reported (Kanter et al. 1986). The quality and quantity of DNA is evaluated from the intensity of ethidium bromide staining after electrophoresis of about 1% of undigested DNA on a 15 cm Yield Gel which includes concentration and size controls.

DIGESTION

DNA is digested with the restriction endonuclease Pst I which recognizes the 6 nucleotide sequence CTGCAG. The initial digestion is performed with a 20 fold excess of enzyme for 1 hour at conditions recommended by the manufacturer. Although 50 nanograms of high molecular weight DNA can yield RFLP results, more evidentiary DNA (about 1 microgram) is usually required because the DNA is often partially degraded. After ethanol precipitation, the cut DNA is resuspended in a small volume and redigested with Pst I. About 10% of this digestion is evaluated on a 15 cm Test Gel including male and female controls. After electrophoresis and staining by ethidium bromide, digested DNA appears as a smear from the top of the gel to below 2 kilobase pairs (kb), similar to the control samples. If the sample is partially degraded, there is less florescence at the top of the lane. The amount of DNA is estimated from the staining intensity relative to the controls.

Electrophoresis and Transfer

The digested DNA is size separated by electrophoresis for three days on a 30 cm Analytical Gel. Size markers composed of bacterial viral DNAs digested with a variety of restriction endonucleases flank the samples and act as a sizing ladder to determine fragment sizes from samples. A control human DNA from cell line K562 is included on each gel at a 100 ng

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concentration. The entire digest from each sample is loaded and lanes are skipped between samples. From a typical rape case the gel is loaded with DNA isolated from victim, evidence, suspect, and whenever possible a single lane containing DNA from evidence and suspect. A piece of numbered nylon mesh is embedded in the gel for identification. The gel is stained with ethidium bromide, photographed, and transferred to a nylon membrane.

Hybridization and Autoradiography:

DNA probes are labelled with 32P and applied sequentially to the membrane to generate the DNA Print. The following polymorphic loci are routinely examined: D2S44, D14S13, D17S79, DXYS14, D18S27, and D14S1. In addition probes which recognize constant regions within the DNA DXZ1 and DNF24, are used to document any interlane mobility differences and the extent of degradation. Hybridizations are performed using standard conditions at 68oC aqueous using oligonucleotide probes. Membranes are washed to 0.1XSSC at 68oC, dried briefly, wrapped in plastic and placed against x-ray film in a cassette for 1 to 5 days to generate autoradiographs. The x-ray film is developed to produce the autoradiograph or autorad (Figure 1). Membranes are stripped of signal with 50% formamide at 68oC and rehybridized (Figure 2).

ANALYSIS

All autoradiographs are reviewed to determine if any overall DNA Prints match visually. If a sample is partially degraded, larger fragments may not be present, for example the D2S44 locus. It has been shown that the DNA Print pattern does not change in degraded samples due to environmental conditions or substrate (McNally et al., 1989A and 1989B). Mobility differences may occur, especially in degraded samples and are documented by the use of probes which recognize monomorphic loci (Figure 3).

POPULATION GENETICS

The frequency of occurrence of the pattern at each locus, except for DXYS14 is calculated by multiplying the allele frequencies of the DNA fragment(s) according to the Hardy-Weinberg equation. All frequencies within plus and minus 1.8% (3 average deviations) of the size of the fragment are added together to determine the allele frequency. For the DXYS14 locus, because haplotypes exist to produce patterns with one to eight bands per person, the database is queried to determine how frequently a particular pattern has been observed. Each of the loci appear to meet the assumptions of Hardy-Weinberg equilibrium because the number of observed and expected combinations appear in agreement. Each locus is on a separate chromosome which is good evidence that there is no linkage disequilibrium. The final frequency of occurrence is calculated by multiplying the individual frequencies of occurrence at each locus. Running the same DNA sample multiple times indicates that sizes fall within 3 average deviations (1 average deviation equals 0.6%

Figure 1

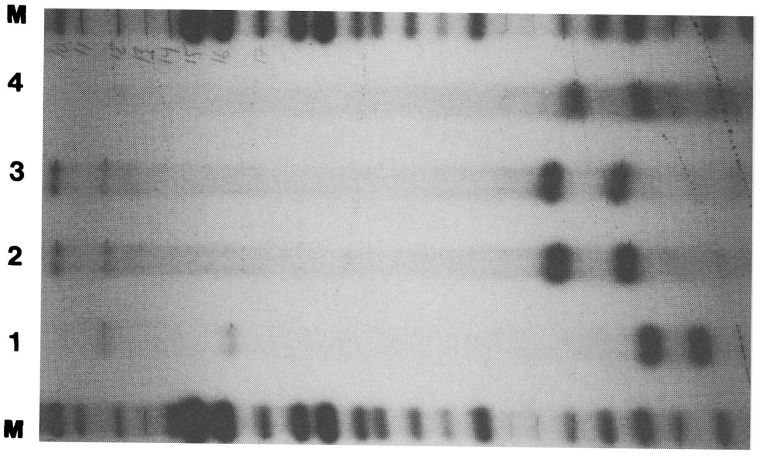
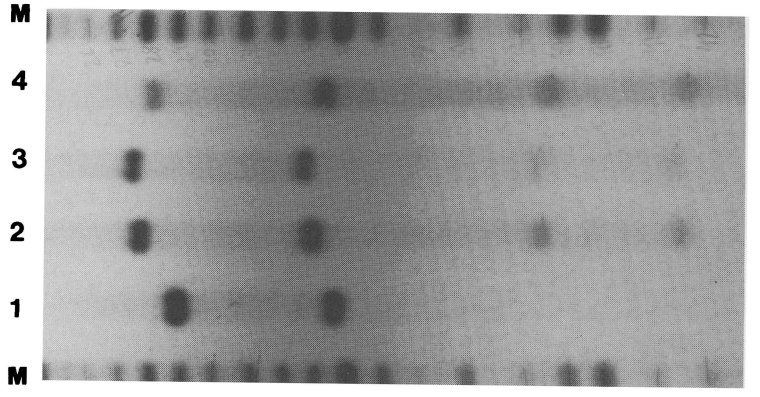


Figure 2



DNA was isolated from the following samples:
20951 - blood from rape victim
20952 - vaginal swab from victim
20953 - semen stain from victim's clothing
20954 - blood from suspect

Figures 1 - 3. Hybridization of the nylon membrane from Gel 1121. Lanes M contain size markers. Lane 1 contains victim. Lane 2 contains vaginal swab. Lane 3 contains suspect. Lane 4 contains semen stain.

Figure 1. Hybridization with probes which detect the highly polymorphic D2S44 and D17S79 loci.

Figure 2. Hybridization with probe which detects the highly polymorphic DXYS14 locus.

Figure 3

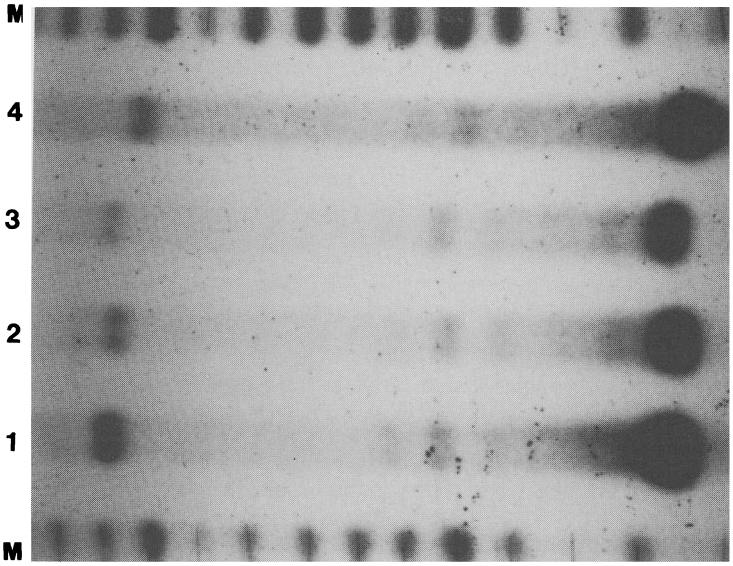


Figure 3. Hybridization with probe which detects the monomorphic DXZ1 locus.

of the fragment size) in a Gaussian distribution. When results of constant bands are included to correct for interlane mobility differences, two fragments are considered to match if they are within 1.8% of each other.

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