DNA Fingerprinting with M13 Probe

A. Leriche

IDNA - Campus Erasme - Route de Lennik, 808 - 1070 - Brussels - Belgium

INTRODUCTION

The genome of vertebrates harbors, within the moderately repeated class of DNA, a large number of relatively short sequences repeated in tandem. These minisatellites show extreme variability in the number of motifs they contain at a given locus. Together with their dispersion throughout the genome, this characteristic has been exploited to generate individual specific DNA fingerprints (1).

In the course of a systematic search for Restriction Fragment Length Polymorphism (RFLP) with the thyroglobulin gene, we observed that the probe corresponding to DNA fragments subcloned in the bacteriophage M13 gave different results when used in the classical hybridization mixture containing Denhardt's solution and herring sperm DNA and the new blotto medium containing dried skimmed milk. In these conditions we observed a complicated and highly polymorphic pattern with the enzyme Hae III. It was evident that this pattern was unrelated to the nature of the insert and that wild-type M13 DNA gave the same results. The logical explanation for this finding was that a segment in M13 hybridized to a hypervariable minisatellite. This particular sequence could be competed for by fish DNA.

FINGERPRINTS IN FORENSIC MEDECINE - RESULTS

We have demonstrated that M13 constitutes an efficient probe to perform DNA fingerprints in man (2) and in animals (3) , provided no competitor DNA is used during hybridization. The patterns obtained are individual-specific and clearly different from those obtained with other probes.

We have determined the probability that two unrelated individuals show the same DNA fingerprint. The value of this probability, called Pc, is 0.27 for the M13 probe which is the same as for the C probe which was obtained by screening a bovine library with M13.

Our technique is derived from the classical Southern blot methodology. After purification, DNA is cut with the restriction enzyme Hinf I. The DNA fragments are separated in an 0.8% agarose gel and transferred to a nitrocellulose membrane.
Prehybridization in formamide 35%, SSCx6, dried skimmed milk 0.25% and EDTA 5 mM is carried out during 4 hours. The hybridization is continued overnight in the same medium containing the ^{32}P-labeled probe. After washing twice at room temperature in SSCx2-SDS 0.1% and twice at 65°C (15 min) in the same medium, the membrane is autoradiographied on X-Ray film with an intensifying screen at -80°C.

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

In forensic medecine, our probe represent a new system with wide application. The DNA fingerprint technique is routinely used in our laboratory for paternity testing. All the cases were resolved without ambiguity with probability of error as low as 1/10000 to $1/10^{7}$ for paternity confirmation and $1/10^{10}$ to $1/10^{18}$ for paternity exclusion. Out of 75 paternity confirmations (= 150 meiosis) no mutation band was detected with the M13 probe.

We have optimized the experimental conditions yielding high molecular weight DNA from different small blood and semen stain collected from different substrates and from different tissues collected from cadavers.

Tissue fragments are frozen in liquid nitrogen, powdered and incubated overnight with proteinase K at 50°C. Sperm cells (after separation of the vaginal cells) and dried blood samples are incubated with proteinase K and β-mercaptoethanol for 2 hours at 50°C. All samples were extracted with 1:1 phenol-chloroform solution (v:v).

All cases of rape we received were resolved following this technique; The different kinds of samples were: vaginal douches or swabs 4 to 24 hours after the rape, dried sperm on clothes and dried sperm mixed with dried blood on clothes.

Identification of suspects by comparison with fingerprint of dried blood samples depends of the nature of the support of the sample. Blood on cotton and nylon support permits extraction of high molecular weight DNA giving interpretable fingerprint.

To confirm the results, all tests were repeated with locus-specific probes as YNH24 . In some cases, we could not obtain a fingerprint with M13 because of problems such restricting the DNA (blood spots, on wood, paint or pavement) but hybridization with YNH24 gave weak but interpretable patterns. In vitro amplification of the DNA using the PCR technique would be helpful for these cases.

REFERENCES:

1: Jeffreys, A.J. et al., Nature, 314 (1985) 67
2: Vassart, G. et al., Science, 235 (1987) 683
3: Georges, M. et al., Cytogen.and Cell Genet., 47 (1988) 127