Volume 16 Number 13 1988 Nucleic Acids Research

Rapid DNA fingerprinting using alkaline phosphatase-conjugated oligonucleotides

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Submitted May 18, 1988

DNA fingerprinting is a powerful technique with applications in genetic mapping, population biology, and forensic medicine (1). By detecting multiple, highly-variable mini-satellite DNA polymorphisms, it is capable of identifying individuals with virtual certainty. Radio-labeled probes, however, limit the use of the technique to research and specialized clinical laboratories. We describe here the use of an alkaline phosphatase-conjugated oligonucleotide probe and colorimetric detection for determining DNA fingerprints.

An alkaline phosphatase-conjugated oligonucleotide corresponding to the core sequence of the human myoglobin 33.6 mini-satellite (1) was prepared by covalent attachment of calf intestine alkaline phosphatase to the 22mer, TGG AGG AGG GCT GGA GGA GGG C, where the underlined thymidine represents the site of attachment (2). By dot-blot analysis, the conjugate could detect one nanogram (approximately 300 molecules) of human genomic DNA.

Human peripheral blood DNA (10μg) is digested with Hinf I or Alu I and electrophoresed on a 1% agarose gel at 20 volts for 16 hours. Following transfer to a nylon membrane and ultraviolet fixation, the blot is prehybridized for 15 minutes in 10 mls of 5X SSC/1% SDS/0.5% BSA at 50°C. Hybridization commences with the addition of the oligonucleotide conjugate to 5nM and the reaction is incubated for 20 minutes at 50°C. The membrane is then washed twice in 1X SSC/1% SDS at 50°C for 5 minutes, twice in 1X SSC/1% Triton X-100 at 50°C for 5 minutes, and twice in 1X SSC at room temperature for 5 minutes. The membrane is developed in low light in 7.5 mls of 0.1M Tris-HCl, pH 9.5/0.1M NaCl/50mM $MgCl_2$ containing 2.5 μg/ml nitro blue tetrazolium and 1.25 μg/ml of 5-bromo-4-chloro-3-indolyl phosphate. Within an hour, bands are visible and in four hours, good resolution of the DNA fingerprint is achieved. If necessary, greater sensitivity can be obtained by overnight incubation.

Figure 1 shows typical results of the above procedure. Five unrelated individuals demonstrate markedly different patterns (lanes a-e), whereas a brother-sister pair (lanes f,g), share several bands consistent with their close relatedness. In another example of this technique, identical twins have the same pattern (lanes h,i). In addition, the same probe gives fingerprints for monkey and rat DNA (data not shown).

The use of an alkaline phosphatase-conjugated oligonucleotide greatly simplifies the technique of DNA fingerprinting. As the conjugate is stable for up to two years, frequent labelling of probes is unnecessary. This non-radioactive technique should allow laboratories to routinely perform DNA fingerprinting analyses without the problems inherent in the use of radio-isotopes.

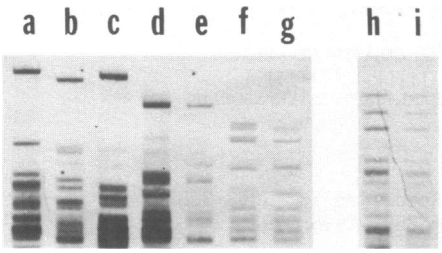

Figure 1: Samples of human peripheral blood DNA were digested with Hinf I, electrophoresed, blotted, and hybridized as described above (only the upper half of the blot is shown). See text for discussion.

ACKNOWLEDGEMENTS

The authors acknowledge Drs. D. V. Santi and L. Marton for advice and encouragement in the course of this work.

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