Rapid, Simple, Non-Isotopic Probing of Southern Blots for DNA Fingerprinting

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INTRODUCTION

DNA fingerprinting, as pioneered by ICI Cellmark Diagnostics using the Jeffreys' probes (Jeffreys et al. 1985, Wong et al. 1987) is a powerful technique for identification in paternity and forensic cases. The technology has previously been based upon ^{32}P. We have developed a non-isotopic alternative using a chemiluminescent enzyme substrate from Lumigen Inc (Schaap et al. 1989). The technology was designed to satisfy the following criteria:- (1) sensitivity - at least equivalent to ^{32}P, (2) simplicity - no additional procedural steps, ideally fewer, (3) sharp bands -to aid interpretation of profiles, (4) sequential probing - at least as convenient as ^{32}P, (5) speed - faster processing time desirable.

METHODS

The probes consisted of the enzyme alkaline phosphatase (as a label) coupled to the 5' end of synthetic oligonucleotides derived from the Jeffreys' minisatellite sequences. Probe synthesis is described in Figure 1. Stability for several months at 4°C has been achieved.

The hybridisation and signal development protocol is described in Figure 2. The rapid hybridisation was facilitated by a high concentration of single stranded probe. The temperature could not be raised significantly above 50°C without inactivating the enzyme label. The room temperature washes removed SDS which interfered with signal generation. Light emission continues for several days so the user is not committed to exposing the film immediately.

RESULTS

Identical results were obtained when conventional ^{32}P probes were compared with the system described above (Fig 3A). Probes could be combined as cocktails without backgrounds increasing (Fig 3B) and high quality multilocus profiles were obtained (Fig. 3C). Filters were able to be re-probed at least 7 times using hot SDS as the stripping agent. Preliminary results on casework filters were presented. By extending the exposure time to 24h with the single locus probe MS1, high molecular weight alleles were still detected with only 10 ng of genomic DNA (Fig 3D). For a 10 kb band in the 50 ng track this represents only 170 fg of target or 10 attomoles of enzyme (assuming 100% hybridisation). Note that in practice much less enzyme remained bound after washing. This was more sensitive than a conventional overnight hybridisation with a purified 4.6 kb insert labelled with ^{32}P by the random hexamer method followed by film exposure for 6 days.

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CONCLUSION

We conclude that the system described exceeds the criteria listed above with superior sensitivity, simpler protocols, sharp bands, easy reprobing and faster processing time. We believe this technology will be the basis of the next generation of DNA fingerprinting tests.

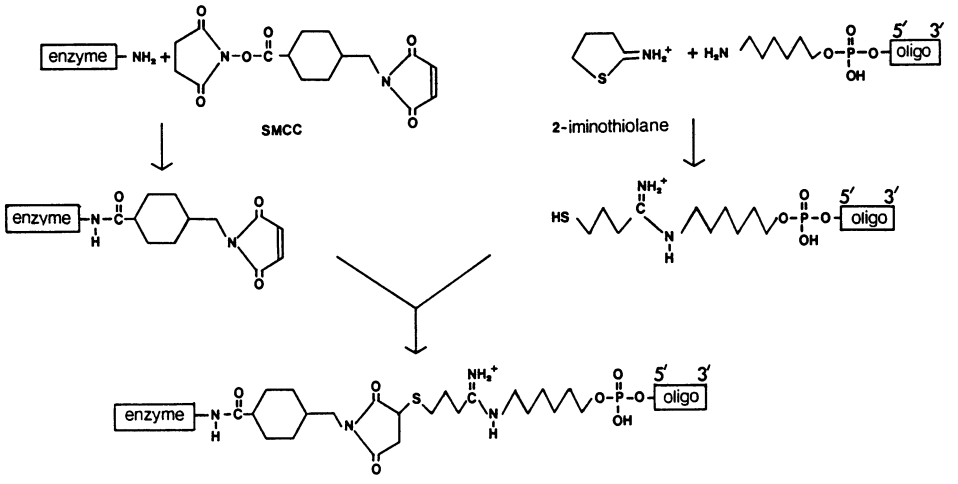

Fig. 1. Synthesis of alkaline phosphatase oligonucleotide conjugates. Aminoalkyl oligonucleotides were synthesised on an Applied Biosystems 380B DNA synthesiser using the reagent "Aminolink 2" (Applied Biosystems). After reaction with iminothiolane, the product was reacted with the maleimido-derivatised enzyme and the resulting conjugate purified by gel filtration on Biogel P-100F (Bio-Rad).

(1) Prepare Southern blot as standard with 2-5 μg Hinf1 digested genomic DNA per track
(2) Prehybridise for 1h at 50°C in 5 x Denhardt's, 5 x SSC, 1% SDS
(3) Hybridise in prehybridisation buffer containing 0.25 nM probe (@ 3 ng/ml) for 20 min at 50°C
(4) Wash 4 x 5 mins at 50°C in 1 x SSC, 1% SDS (multilocus probes) (for single locus probes the last 2 washes contained 0.25 x SSC)
(5) Wash 2 x 5 mins at room temperature in 1 x SSC
(6) Apply substrate, place filter in cassette with film and leave for 2 h at 37°C
(7) Develop film

Fig. 2. Hybridisation and signal development protocol.

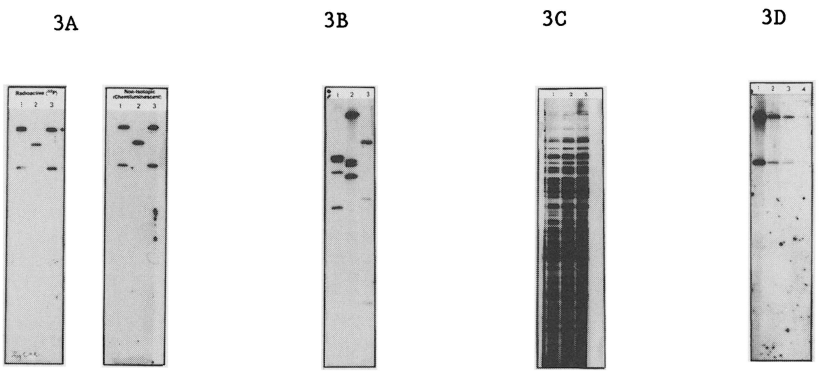

Fig. 3A. Comparison of ^{32}P with non-isotopic system. The non-isotopic system used the protocol described in Fig. 2. The same filter was subsequently probed with ^{32}P using the random hexamer method to label a purified 6.3 kb insert followed by overnight hybridisation and 6h film exposure. Probe MS43 was used.

Fig. 3B. Use of a cocktail of two single locus probes. The protocol was as described in Fig. 2 except that two probes were used simultaneously, each at 0.25 nM. Probes MS1 and MS31 were used. Tracks 1-3 represent different individuals.

Fig. 3C. A multilocus fingerprint using chemiluminescence. The protocol was as described in Fig. 2. Probe 33.6 was used. Tracks 1, 2 and 3 represent genomic DNA loadings of 2, 4 and 6 μg respectively.

Fig. 3D. Sensitivity with a single locus probe. The protocol was as described in Fig. 2 except the exposure time for the film was increased to 24h. Tracks 1-4 represent genomic DNA loadings of 500, 100, 50 and 10 ng respectively.

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