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DNA fingerprinting using non-radioactive oligonucleotide probes specific for simple repeats

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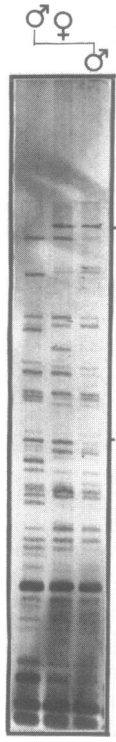

Fig.: Hinf I-digested DNAs of the parents and their child was electrophoresed, blotted and hybridized to the digoxigenin-labeled $(CAC)_5$ oligonucleotide probe.

Using probes recognizing ubiquitously interspersed simple repetitive sequences many independent loci can be detected simultaneously (1,2). In order to allow broad application of DNA fingerprinting it is necessary to establish a simple, fast, sensitive and highly reproducible, non-radioactive detection method. For this purpose the oligonucleotides $(CAC)_5$ and $(GACA)_4$ were synthesized by the solid phase phosphoramidite method. Aminolink 1™ (Applied Biosystems) or a hexylamin linker (New Brunswick Scientific) were coupled like an additional base to the 5' end. Digoxigenin-amidocaproic acid-N-hydroxysuccinimidester was covalently attached following a modified protocol as described for biotinylation (3). The oligonucleotides were purified by RP18-HPLC. Hinf I-digested human DNA was electrophoresed in 0.7% agarose gels, alkaline-blotted onto Immobilon™ PVDF membranes (Millipore) and hybridized as described previously (3; $(CAC)_5$ concentration 10 pmoles/ml; more intense signals were obtained by lowering the hybridization temperature 3-5°C as compared to ^{32}P-labeled oligonucleotides). Digoxigenin-labeled oligonucleotides were detected colorimetrically using an alkaline phosphatase-conjugated antibody specific for digoxigenin (BOEHRINGER; 0.9 M NaCl; agarose gel overlay; substrates: BCIP, NBT). Strong bands are visible in 2 to 3 minutes, the complete banding pattern after several hours (Fig.). The color precipitate can be stripped by dimethylformamide and the membrane rehybridized. In contrast to hybridizations with biotinylated probes, this procedure is always reproducible and background can easily be controlled by duration of development. Probes are stable for months (4°C) and can be reused many times.

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