Application of simple repeat oligonucleotides for DNA fingerprinting

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INTRODUCTION

Simple repetitive DNA sequences consist of short, tandemly repeated sequence motives, each motif consisting of up to ten bases, e.g. the dimeric ..CTCTCTCT.. or the quadruplet ..GATAGATAGATA.. The uninterrupted simple sequence stretches are in general some 50-200 (-500) nucleotides long and they are generally spread all over the chromosomal complement (Epplen 1988). Besides the "perfect" tandem repetitions, often there are also "degenerating" elements found in the genome and mixtures like $(GT)_{25}(GA)_{15}$. Residing in intergenic spacers and introns, certainly most of the simple elements do not exert any present-day sequence-dependent function und thus they do neither contribute to the phenotype nor even to the behaviour of a given organism. Because of their peculiar structure, simple sequences are prone to a number of mutational events, only two of which are mentioned here: i) Slipped strand mispairing probably generates variant numbers of individual sequence motives, while ii) unequal crossing over mutations result in large scale length variations. The former changes can be demonstrated in high resolution denaturing sequencing gels after molecular cloning (cave cloning artifacts of "poisonous" repetitive sequences in prokaryotes!) or better by direct genomic DNA amplification via the polymerase chain reaction. The latter long range mutations are more easily shown, e.g. by conventional agarose gel electrophoreses and subsequent probe hybridizations. Both methods proved the hy-pervariability of simple sequences (for review see Epplen et al. 1989). In summary their overall biological meaning may be best understood by regarding simple sequences as "the consequences of the properties of eukaryotic genomes". For the purpose of this discussion we shall restrict ourselves exclusively to the use of simple repetitive DNA as a fool-proof tool. Since all eukaryotes contain different sorts of simple repetitive DNAs interspersed in their genomes, it seemed reasonable to try to device a generally applicable method for the simultaneous multilocus detection, preferably by oligonucleotides. This allows to generate highly informative, individual-specific hybridization patterns in man (Nürnberg et al. 1989), in all investigated animal as well as plant species (Epplen et al. 1989).

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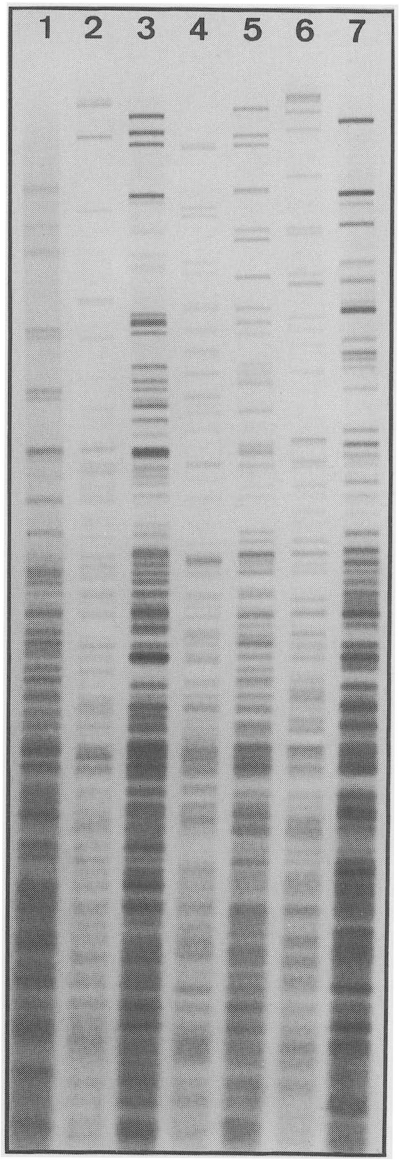
ON THE METHODOLOGY OF NON-RADIOACTIVE OLIGONUCLEOTIDE FINGERPRINTING IN THE GEL



In order to continously simplify and speed up the routine fingerprinting pro-cedure, already initially we obstained from time-consuming Southern-blotting onto expensive membranes and hence hy-bridized the ^{32}P-labeled oligonucleotide probes directly in the gel. For man $(CAC)_5$ or the complementary $(GTG)_5$ ren-der identical, highly informative fin-gerprints. Meanwhile also the exact per-centage of mutated bands has been deter-mined in a large number of meioses (Nürnberg et al. 1989). Satisfying non-radioactive oligonucleotide fingerprint patterns, however, were then demon-strable only after DNA transfer onto PVDF membranes (Zischler et al. 1989a). Most recently both advantages could be combined (see preliminary report by Zischler et al. 1989b as well as the Figure on the left): Here about 10 ug of DNA from seven healthy, unrelated volunteer donors was digested with the restriction enzyme Hinf I and electro-phorezed in a 0.7% agarose gel in TBE buffer; the gel was dried and hybridized with the digoxigenated oligonucleotide $(CAC)_5$ as described (op. cit.). After extensive washing in 0.9 M NaCl at room temperature, 1 ml of a solution was spread onto the gel containing the mono-specific anti-digoxigenin antibody coupled to alkaline phosphatase (BOEH-RINGER-Mannheim, FRG) in a 1:500 dilu-tion for 3 h (37°C). The gel was laid overnight onto developing agar con-taining NBT and BCIP. Hybridization signals were recorded as sharp purple-blueish bands using AGFA-Ortho film. Note the individual-specific hybridi-zation patterns in each lane. Intensity differences between the samples result from the unequal amounts of DNA loaded. In a more carefully controled serial dilution experiment it was shown, that 4-5 ug of human DNA are absolutely sufficient for the non-radioactive fingerprint analysis (data not shown).

ON THE PRACTICAL APPLICATIONS

Because of the severely restricted space available, the whole range of applications and particularly the advantages of oligonucleotide fingerprinting cannot be addressed here adequately. Suffice it to say that in addition to fingerprinting purposes simple repeat oligonucle-otide hybridization in situ e.g. to metaphase chromosomes (Zischler

et al. 1989) opens a new avenue to study the evolution of genomic and chromosomal organization. Due to the DNA stability and the information content the superiority of fingerprints over conventional marker serology has already been demonstrated in actual case work (Roewer et al. 1989; Sprecher et al. in press). Different oligonucleotides are available depending on the specific problem to be solved. Yet the whole subject of stain analyses by oligonucleotide fingerprinting has to be covered in more detail elsewhere (Roewer et al. to be submitted). Finally in addition to paternity and forensic questions many other problems in a variety of medical and veterenary disciplines can now be tackled with ease and unprecedented precision: zygosity determination in twins, genomic changes in tumours (Lagoda et al. 1989), chimaerism after bone marrow transplantation, semen identification, genetic relationship analysis, more effective animal and plant breeding programs. Nevertheless it should always be remembered, that fingerprints from simple repetitive sequences reflect only the length information of some 10-50 DNA fragments originating from "the barren stretch of desert" around the few "gene oases" in eukaryote genomes.

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