An 18 mer sequence in a rat 1.3 kbp *Eco*RI repeat detects genetic polymorphism in humans

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DNA fingerprinting involves the typing of an individual's DNA content to produce somatically stable, individual-specific DNA fingerprints. This technique often uses hypervariable minisatellite (HVMS) sequences as the fingerprinting probe and has found extensive use in several disciplines. Recently, we sequenced a 1.3 kbp *Eco*RI repetitive DNA fragment, shown to harbour the meiotic DNA repair site(s) of rat pachytene spermatocytes. This 1.3 kbp clone contained four sequences sharing high homology to the various HVMS sequences reported in the literature. Here we show that one of the sequences can indeed detect polymorphism in human individuals and can be used for DNA fingerprinting.

HYPERVARIABLE minisatellite (HVMS) sequences are highly prevalent in eukaryotic genomes of a number of species including humans. Minisatellites consist of short G+C repeats present in tandem to form arrays. They display strand asymmetry, in that one strand has a high G content[1]. Though no overall sequence consensus has been noted, several families of minisatellites identified contain a consensus 'core' sequence of 10 to 15 bp (ref. 2). Minisatellite sequences display considerable polymorphism in terms of the number of repeats present in an array and also in the sequence composition of each individual repeat within the array. Taking advantage of the genetic polymorphism detected by these sequences at several loci in the genome, Jeffreys and coworkers[2,3] developed the principle of DNA fingerprinting. This technique initially utilized the core sequences of HVMS as probes to generate somatically stable, individual-specific DNA fingerprints. More recently, Ehtesham *et al.*[4] developed a novel probe for human DNA fingerprinting which contained chi-like sequences. DNA fingerprinting has found wide-spread application in several disciplines including forensics, paternity testing, ecological genetics, immigration laws and transpiant screening to name a few. Over the years, this technique has undergone considerable refinement. Polymerase chain reaction (PCR) amplification of hypervariable loci, has considerably increased the sensitivity of DNA typing systems and has proved extremely useful when the DNA source is limiting or degraded[5].

The mechanism of generation of polymorphism has generated considerable debate over the last several years. Owing to the high homology of the 'core' sequence of the HVMS with that of the general recombination signal of *E. coli* (chi) it has often been postulated that these

sequences could be the eukaryotic initiators of recombination promoting among other things their own propagation[2]. Direct evidence for this hypothesis is, however, lacking. Debate on whether the propagation is due to germline or somatic events also exists. While similar minisatellite mutation rates in male and female germ cells suggest that hypervariability is a consequence of meiotic events[6], detection of new mutant minisatellite alleles, in early mouse development indicates that mutation events can also arise during mitosis[7].

Over the past several years, we have been studying DNA repair synthesis at the pachytene interval of meiosis in rat pachytene spermatocytes with an aim of understanding the significance of this event in the context of the events at this stage of meiosis mainly recombination.

Recently, we have analysed the meiotic DNA repair sites of rat pachytene spermatocytes and sequenced a member of the 1.3 kbp *Eco*RI repetitive DNA family (1.3 A), found to harbour the meiotic DNA repair sites[8]. The sequence contained (a) a $(CAGA)_6$ repeat, a $(CA)_{22}$ repeat, and (c) four sequences showing a high percentage of homology to the various HVMS sequences reported. Among these four sequences was an 18 mer sequence (5′ GGGAGGGAGTGAGGATTG 3′) sharing a 90% homology to a core sequence (GGNNGTGGGG) derived from a comparison of DNA sequences of several variable number tandem repeat loci described by Nakamura *et al.*[9], a 69% homology to the myoglobin core and the human consensus minisatellite core[2] and a 63% homology to the mouse MHC recombination hotspot[10] (Figure 1). In order to evaluate the significance of the HVMS sequences in the repair positive clone, we were interested in determining whether this sequence could detect genetic polymorphism in humans. Here we show that this sequence can indeed detect polymorphism in human individuals and could be used for DNA fingerprinting in the human population.

Genomic DNA was isolated from the peripheral blood of four related donors (consisting of a father, mother and a pair of identical twins) and four unrelated donors.

Myoglobin core (2)	GGAGGTGGNCAGGRRG
Rat 1.3A	GGAGGAGTGAGGATT
Nakamura core (VNTR) (9)	GGNNGTGGGG
Rat 1.3A	GGGAGTGAGG
Human consensus minisatellite core' (2)	GGAGGTGGGCAGGAXG
Rat 1.3A	GGAGGGAGTGAGGATT
Mouse MHC recombination hotspot (10)	GGAGGTAGGCAGGCAG
1.3A	GGAGGGAGTGAGGATT

he rat 1.3 A HVMS-like sequence with 'NTR and recombination hotspot sequences.

Following digestion of the genomic DNA with *Hinf*I (which does not cut within the minisatellite sequences), the DNA samples were run on a 1% agarose gel and transferred to nylon membrane (gene screen plus du pont). The 18 mer oligonucleotide was end labelled with γ-^{32}P-ATP using T4 polynucleotide kinase. The nylon membrane was prehybridized for 30 min at 37°C in prehybridization solution (6 × SSC, 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg ml^{-1} yeast tRNA and 0.5% sodium dodecyl sulphate). Hybridization was carried out for 30 h at 37°C in hybridization solution (6 × SSC, 5 × Denhardt's solution, 0.05% sodium pyrophosphate, 100 μg ml^{-1} yeast tRNA) containing 1×10^6 cpm ml^{-1} of the end labelled probe. Yeast tRNA has been used as the non-specific nucleic acid instead of salmon sperm DNA, to prevent any non-specific titering out of the probe. As is evident in Figure 2, the identical twins had identical fingerprints and most of the bands identified could be traced to one or the other parent. At the same time, the DNA samples from the four unrelated individuals showed hybridization patterns, each distinct from the other. Thus, it is clear that the 18 mer sequence from the rat *Eco*RI 1.3 kbp family can detect polymorphism in the human population. It may be pertinent to point out here that recently Mazzarella *et al.*[11] demonstrated that using PCR technique and syntenically equivalent tagged sites from human DNA, one can assemble corresponding genomic maps from other primates as well as rodents. Although the detection of polymorphism in humans by rat HVMS-like sequence is not that surprising, we would like to stress here that the availability of the oligonucleotide probe described in this study would be very valuable for DNA fingerprinting studies in Indian population.

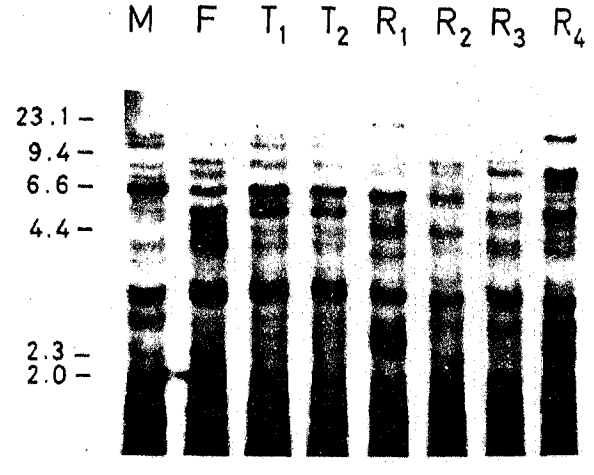

Figure 2. *Hinf*I digested human genomic DNA hybridized with 18 mer rat sequence. Genomic DNA samples were obtained from four related donors—mother (M), father (F) and a pair of identical twins (T_1, T_2) and four unrelated donors, R_1, R_2, R_3, R_4.

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