A Multi-locus Probe for Human DNA Fingerprinting Based on *chi*-like Sequences

Nasreen Z. Ehtesham* and Seyed E. Hasnain#

National Institute of Immunology, New Delhi 110067, India

INTRODUCTION

DNA fingerprinting probes based on Jeffreys minisatellite core or those exhibiting similarities with this G-rich core consensus (GGGCAGGAXG), such as the $(GTG)_5$ simple repeat (Schafer et-al 1988) or random G-rich oligodeoxynucleotides (Vergnaud 1990) presumably corresponding to VNTRs, share a degree of homology with the bacterial recombinator activator signal (*chi*), which is believed to serve as foci for recombination (Jarman and Wells 1988, Murray et-al 1988).

A synthetic oligodeoxyribonucleotide containing *chi*-like sequences was designed on the assumption that hypervariability is partly due to the presence of molecular signals which promote recombination. The utility of this probe in DNA fingerprinting is described.

RESULTS AND DISCUSSION

We designed an oligo, O-*chi*-1 (Ehtesham et-al 1991) containing novel *chi* homologues (GGAGGAGG). Our *chi* was based on sequences naturally implicated in recombination, leading to genetic variability (Table 1). A sequence resembling *chi* is also found in immunoglobulin gene rearrangements, glycine-rich plant stress proteins etc. O-*chi*-1 was chemically synthesized on the basis of the assumption that hypervariabilty is partly generated due to recombination events wherein these *chi*-like sequences are involved. A probe comprising of *chi*-like sequences when used for DNA fingerprinting should, therefore, generate individual specific fingerprints.

Human DNA was digested with different restriction enzymes, Southern transferred and probed with the 33 base long radiolabeled O-*chi*-1. Individual-specific DNA fingerprints were obtained even under extremely high stringency of hybridization and washing (6x SSC/0.2 % SDS, 65°C, 4 h). The number of bands per individual varied from 11-18, between 4-25 kb range, with an average number of 15.1 bands. Band sharing frequency data were obtained by a comparison of DNA.

* Present address: Plant Molecular Biology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, 110067, India; # Corresponding author

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

Table 1. Sequences implicated in naturally occurring processes

	Sequence	Reference
O-*chi*-1	(GGAGGAGG)*	
E. coli chi	(GCTGGTGG)	
Minisatellite consensus	(GGGCAGGAXG)	Jeffreys et-al 1985.
Myoglobin *chi* homologue	(GCAGGAGG)	Krowczynska et-al 1990.
Mouse MHC recombination hotspot	(GGAGGTAG)	Steinmetz et-al 1986.
Homologous recombination hotspot	(AGAGGTGG)	Wahls et-al 1990.
Hamster *aprt* insertion/deletion locus	(CCAGGAGG)	Meuth et-al 1987.
Breakpoints of oncogene *bcl2* translocations	(GCAGGAGG)	Krowczynska et-al 1990.

*bases identical to *chi* are underlined

band patterns of unrelated individuals and parent-child combinations. The banding pattern in two individuals was compared by using the statistical calculation $D = 2N_{AB}/(N_A + N_B)$ where N_A and N_B are the number of bands in individual A and B, and N_{AB} is the number of common bands between individuals A and B (Schacker et-al 1990, Wetton et-al 1987). The band sharing frequency (*D*) data are presented in Table 2. Compilation of similar calculations from DNA fingerprints of a number of other individuals gives the band sharing frequency value for O-*chi*-1 to be 14.2 ± 2.7 % for unrelated individuals and 55.4 % for parent offsprings. The probability that all the bands in one individual

Table 2. *D* value calculations for O-*chi*-1, based on DNA fingerprints of nine different individuals#

AB	.167							
BN	.190	.286						
KS	.083	.167	.190					
SH	.071	.000	.080	.071				
EH	.071	.072	.160	.143	.563			
NH	.000	.000	.000	.000	.303	.545		
ND	.067	.067	.074	.015	.177	.235	.171	
AD	.067	.067	.074	.015	.177	.235	.171	1.00
	MG	AB	BN	KS	SH	EH	NH	ND

#Note the *D* values for parent-offsprings (SH/NH *vs* EH), distant degree cousins (SH *vs* NH), and homozygotic twins (AD *vs* ND)

will be common to another unrelated individual was $14.2^{15} = 1.9 \times 10^{-13}$. This value compares very well with the reported values (9×10^{-6} to 2×10^{-8}, depending upon the restriction enzyme used) for simple repeats (Schafer et-al 1988) or with that of $2X10^{-22}$ for a combination of the extensively used 33.15 and 33.6 minisatellite probes (Jeffreys and Morton 1987). It is significant to mention that the probability for O-*chi*-1 could further go up if fingerprinting is carried out on DNA digested with different restriction enzymes.

A closer look at Table 2 further reveals that the parents (SH and NH) of the individual (EH) also shared DNA bands between themselves - a consequence of inbreeding phenomenon fairly prevalant in certain social/caste systems in India.. This family was deliberately selected for this analysis in order to determine if O-*chi*-1 probe could also detect varying degrees of relationships between individuals. A further application of this probe in determining twin zygosity is also illustrated in Table 2. Genomic DNA was

isolated from a pair of twins who presented an unusual clinical case. These twins were connected to the same placenta but by two separate umbilical chords. In usual homozygotic twins the umbilical chord is shared, whereas in heterozygotes there are two different placentae. Fingerprinting of these twins besides solving the clinical riddle, also indicated the utility of O-*chi*-1 in determining zygosity. A large number of exactly identical DNA bands were obtained for the twins, thus indicating their homozygotic nature. It is therefore, apparent that this probe can be also used to estimate the degree of relatedness besides paternity testing and zygosity determination.

ACKNOWLEDGEMENT

We thank Dr. Om Singh for biostatistical analyses. NZE was a Fellow of the Talwar Research Foundation, New Delhi, India.

REFERENCES

Ehtesham NZ, Das A and Hasnain SE (1991) A novel probe for human DNA fingerprinting based on *chi*-like sequences. Gene 00:000 (In Press)

Jarman AP and Wells RA (1988) Hypervairable minisatellites: recombinators or innocent bystanders? Trends Genet 5:367-371

Jeffreys AJ and Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18:1

Jeffreys AJ, Wilson V and Thein SL (1985) Individual specific fingerprint of human DNA. Nature 316:76-79

Krowczynska AM, Rudders RA and Krontiris TG (1990) The human minisatellite consensus at breakpoints of oncogene translocations. Nuc Acids Res 18:1121-1127

Meuth M, Nalban-tolfu J, Pheaar D and Miles C (1987) Molecular basis of genome rearrangements at the hamster aprt locus: In: Banbury Report 28: Mammalian Cell Mutagenesis, Cold Spring Harbor Laboratory, 183-191

Murray MJ, Haldeman BA, Grant FJ and O'Hara PJ (1988) Probing the human genome with minisatellite like sequences from the human coagulation factor VIII gene. Nuc Acids Res 16: 4166

Schafer R, Zischler H and Epplen JT (CAC)5, a very informative oligonucleotide probe for DNA fingerprinting. Nuc Acids Res 16: 5196

Schacker V, Schneider PM, Holtkamp B, Bohnke E, Fimmers R, Sonnenborn HH and Rittner C (1990) Isolation of the minisatellite probe MZ 1.3 and its application to DNA fingerprinting analysis. Forensic Sci Internatl 44:209-224

Steinmetz M, Stephan D and Lindahl KF (1986) Gene organization and recombinational hotspots in the murine major histocompatibility complex. Cell 44:895-904

Vergnaud G (1989) Polymers of short oligonucleotides detect polymorphic loci in the human genome. Nuc Acids Res 17:7623-7630

Wahls WP, Wallace LJ and Moore PD (1990) Hypervariable minisatellite DNA is a hotspot for homologous recombination in human cells. Cell 60:95-103

Wetton JH, Carter RE, Parkin DT and Walters D (1987) Demographic studies of a wild house sparrow population by DNA fingerprinting. Nature 327:147-149