

FREQUENCY DATABASES FOR THE DNA PROBES MS1, MS31, MS43A, AND YNH24, DERIVED FROM CAUCASIANS, AND AFRO-CARIBBEANS IN THE LONDON AREA

C. Buffery, F. Burrige, M. Greenhalgh, S. Jones, and G. Willott

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London, United Kingdom, SE1 7LP

INTRODUCTION

DNA from blood samples submitted to Metropolitan Police Forensic Science Laboratory have been analysed with the probes MS1, MS31, MS43A and YNH24 using the restriction enzyme Hinf I. Frequency databases have been prepared from more than 1000 Caucasian and more than 500 Afro Caribbeans in order to assess the evidential significance of matching DNA profiles.

METHOD

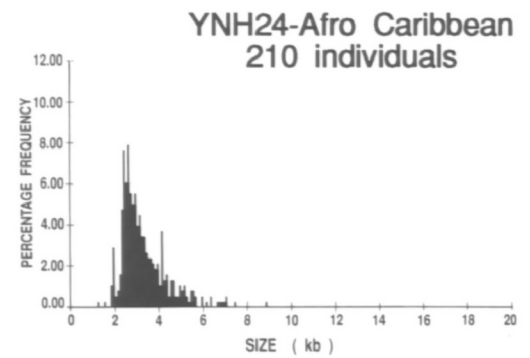
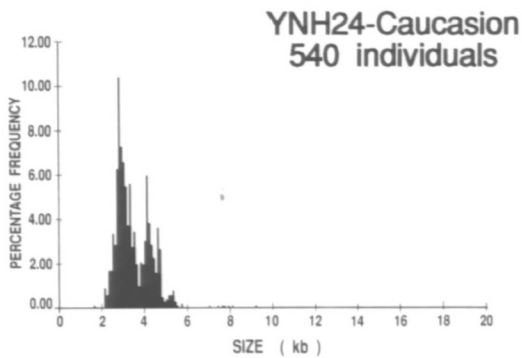
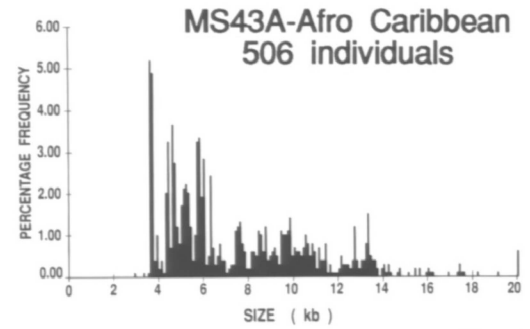
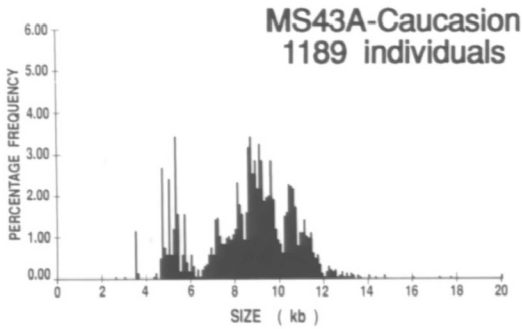
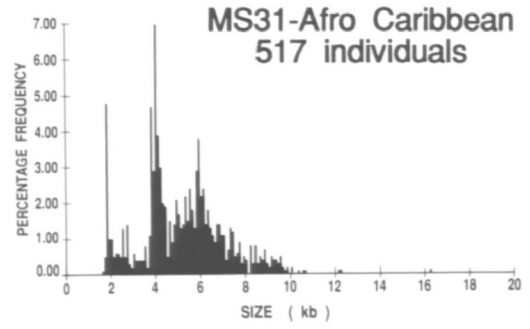
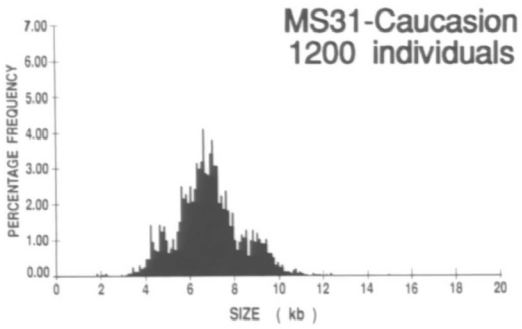
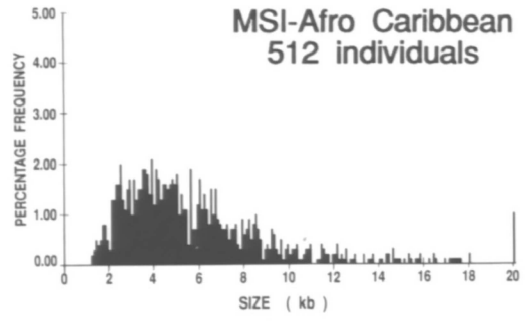
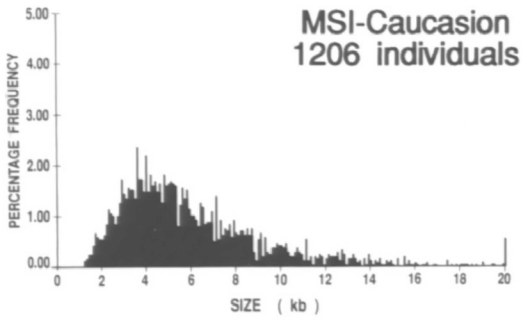
The protocol used was developed in this laboratory and is based on the method of Smith (1990) and Gill (1987, 1990) with certain modifications.

DNA was extracted from 150 μ l aliquots of liquid blood. Phenol and phenol/chloroform extractions were performed to purify the DNA. Following a partial restriction stage the DNA was then quantified using a Hoefer TKO100 DNA fluorometer. Complete restriction of 500ng DNA (typically 1-3 μ l) was carried out with Hinf I at 37°C overnight. Pre- and post-restriction samples were analysed on a 1% agarose minigel to test the quality of the extracted DNA and the efficiency of restriction. Samples showing incomplete restriction were further purified and re-restricted.

Size separation of restricted DNA was achieved by electrophoresis in a 0.7% agarose gel running in TBE buffer at 70 volts. Electrophoresis continued until a 2.3 kb λ /HindIII fragment had migrated 15cm from the origin (approximately 18 hours).

Following denaturation the DNA was transferred onto a nylon membrane using a vacuum transfer apparatus. DNA was fixed by baking the membrane at 80°C for 2 hours or ultra-violet irradiation.

DNA probes MS1, MS31, MS43A (Cellmark Diagnostics) and YNH24 (Promega Corporation) were radiolabelled by ³²P dCTP by the random priming technique using Amersham's oligo-labelling kit. Unincorporated nucleotides were removed by gel filtration through a 1ml Sephadex G50 column. Using a modified Church and Gilbert protocol (1984), prehybridisation for 5 minutes was followed by hybridisation with radiolabelled probe at 65°C overnight. The membranes were rinsed with 2xSSC, 0.1% SDS followed by longer washes with 0.1xSSC, 0.1% SDS. Autoradiography was carried out at -70°C for 1 to 10 days. Membranes were stripped of probe before re-hybridisation with another.



**Distribution of MSI, MS31, MS43A and YNH24 alleles
For Caucasians and Afro Caribbeans in the London area
(Histograms reproduced here by kind permission of Forensic Science International)**

All autoradiographs were analysed using a video-based scanning system developed in this laboratory (Catterick et al. 1991; Buffery et al. 1991) which utilises the Elder and Southern (reciprocal method, local form [1987]) for band size calculation with reference to molecular weight markers at three differing positions on the autoradiograph. Data points were recorded at 0.01 kb intervals and binned to 0.1 kb for generation of the histograms.

RESULTS AND DISCUSSION

Careful quantity estimation of purified DNA resulted in uniform band intensities which ensures greatest accuracy and reproducibility of sizing using the video scanning system.

The histograms generated by our data show good correlation with previously published, smaller data collections where loci and restriction enzyme are in common (Smith et al. 1990; Gill et al. 1991; Brinkman et al. 1991; Odelberg et al. 1989).

The major ethnic groups do demonstrate differences in their allele distributions but alleles of exceptionally high frequency have not become apparent.

Interestingly, MS1 which has the highest observed mutation rate (0.052 per gamete [Jeffries et al. 1988]) displays the least variability between ethnic groups. Hence MS1 is considered to be an extremely powerful forensic probe and is likely to be less sensitive than other loci to the effects of population substructure.

References

- Brinkmann B, Rand S and Wiegand P (1991) Population and family data of RFLP's using selected single and multilocus systems. *Int. J. Leg. Med.*, 104: 81-86
- Buffery C, Catterick T, Greenhalgh M, Jones S and Russell JR. (1991) Assessment of a video system for scanning DNA autoradiographs. *Forensic Sci. Int.*, 49: 17-20
- Catterick T and Russell JR (1991 in press) The Development of a video scanner for forensic DNA autoradiographs. *Lab. Microcomputer*
- Church GM and Gilbert W. (1984) Genomic Sequencing. *Proc. Natl. Acad. Sci. USA*, 81: 1991-1995
- Elder JK and Southern EM (1987) Computer aided analysis of one dimensional restriction fragment gels. In: Bishop MJ and Rawlings CJ (eds) *Nucleic acid and protein sequence analysis*. IRL Press, Oxford, pp 165-172
- Gill P, Lygo JE, Fowler SJ and Werrett DJ. (1987) An evaluation of DNA fingerprinting for forensic purposes. *Electrophoresis*, 8: 38-44
- Gill P, Woodroffe S, Lygo JE and Millican ES. (1991) Population Genetics of Four Hypervariable Loci. *Int. J. Leg. Med.*, (in press)
- Gill P, Sullivan K and Werrett DJ. (1990) The analysis of hypervariable DNA profiles: problems associated with the objective determination of the probability of a match. *Hum. Genet.*, 85:75-79
- Jeffreys AJ, Royle NJ, Wilson V and Wong Z. (1988) Spontaneous mutation rates to new length alleles at tandem repetitive hypervariable loci in human DNA. *Nature*, 332: 278-281
- Odelberg SJ, Plaetke R, Eldridge JR, Ballard L, O'Connell P, Nakamura Y, Leppert M, Lalouel JM and White R. (1989) Characterization of eight VNTR Loci by Agarose Gel Electrophoresis. *Genomics* 5: 915-924
- Smith JC, Newton CR, Alves A, Anwar R, Jenner D and Markham AF. (1990) Highly polymorphic minisatellite DNA probes. Further evaluation for individual identification and paternity testing. *J. Forensic Sci. Soc.*, 30: 3-18