

Allele frequencies distribution of two VNTR markers (YNH24; YNZ22) in PST I digests from random Italian individuals (population of Rome)

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INTRODUCTION

Since the first report of a highly polymorphic locus in Man (Wyman and White, 1980), numerous hypervariable DNA sequences have been described. These markers have been given the name VNTR (Nakamura et al, 1987), and represent nowadays a major tool in forensic biology and paternity testings.

The most systematic investigation on human VNTR has been carried out by Nakamura et al (1987, 1988) who used synthetic oligonucleotides (reproducing core sequences of known hyperpolymorphic loci) for screening a genomic cosmid library. Seventy-two VNTR were this way discovered. Among these, YNH24 (D2S44) and YNZ22 (D17S30) proved to be very polymorphic over several genomic digests (as PSTI, TaqI, HinfI).

Prior to introducing these markers in the routine of the forensic applications, their formal and population genetics need to be studied further.

Here we report the allele frequencies distribution of YNH24 and YNZ22 in about 80 random individuals from Central Italy (Rome). This is our first contribution to a collaborative project for the creation of a repertory of VNTR frequencies in the population of Italy.

MATERIALS AND METHODS

Eighty PSTI genomic digests were prepared according to standard procedures (Maniatis et al., 1982). Seven μ g of each digest were run on agarose (0.7% W/V) gels (250 x 150 X 4 mm) using a submarine electrophoretic set. Separations (70 mA /gel) were prolonged as far as the 4 Kb of lambda DNA/Hind III digest marker had migrated beyond the middle of the gels. After capillary Southern transfer on nylon membranes, the resulting filter blots were hybridized overnight under high stringency conditions (Church and Gilbert, 1984). A rotating incubator conditioned to 65 C was used with filters rolled into plastic cylinders. Prehybridization with competitor DNA (250 μ g/ml for 17 h in the same buffer used for subsequent hybridization) was necessary for YNZ22 only. Cosmid-inserted probes (100 ng) - kindly supplied by Dr. Y. Nakamura, amplified in HB101 host cells and extracted by alkaline lysis

minipreps - were labeled with ^{32}P dCTP by a random exanucleotide priming method. Filters were finally washed at 65 C for 15' with 3xSSC 5%SDS, 15' with 1xSSC 1%SDS, and 15' with 0.1xSSC 0.1%SDS. After rinsing in 2xSSC they were finally exposed for autoradiography (1-6 days).

Mobilities of the restriction fragments was independently measured on the original Xray films by two persons of our staff, using a hand ruler. Allele sizes were then calculated using lambda/HindIII digest bands as reference positioned at the side extremities of each gel. A simple computer program based on algorithms by Elder and Southern (1983) led us convert mobility into fragment length values. Gels always contained digests of two selected individuals whose Kb values made us calculate standard deviations and percentages of error in size assignment to alleles of both systems. An average 2% error in alleles size assignment was found for both systems. The maximum resolution in the gels was extrapolated using the nearest bands still discernible in heterozygous samples (1mm corresponding to 250 bp on average for YNH24, and to 50 bp for YNZ22).

RESULTS AND DISCUSSION

Values reported above (% error, maximum resolution in gels) were used to plot the following graphs reproducing the distribution of alleles (Fig.1).

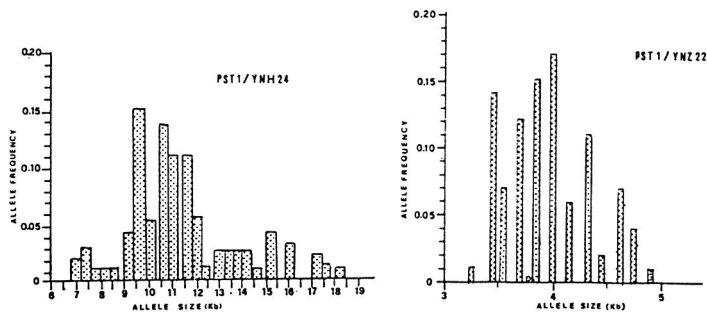


Fig.1. Distribution of YNH24 (left, 73 digests) and YNZ22 (82 digests) alleles

In Table 1, average allele frequencies (\bar{q}), heterozygosity (H) and inclusion probability $[(q^2 (2-q))]$ and probability for a given allele to be fortuitously present in an unrelated individual $[(2q - q^2)]$ are reported.

By estimates reported here, YNH24 and YNZ22 are somewhat less polymorphic with PSTI than with other restriction enzymes. However, as shown in Fig.2, band patterns of the two systems do not interfere each other with PSTI digests, so that a

Table 1. Statistical inferences on PstI/YNH24 - YNZ22

	alleles	\bar{q}	H	$q^2 (2-q)$	$2q - q^2$
<u>YNH24</u>	23	0.15	0.85	0.042	0.28
<u>YNZ22</u>	12	0.23	0.77	0.094	0.41
<u>comb.</u>	-	-	-	0.0038	0.11

combined typing with a probe mix in just one hybridization should be easily performed in casework analyses. Due to this fact, and thanks to the yet high discrimination power of the two systems, PSTI/YNH24-YNZ22 is a suitable protocol for paternity investigations.

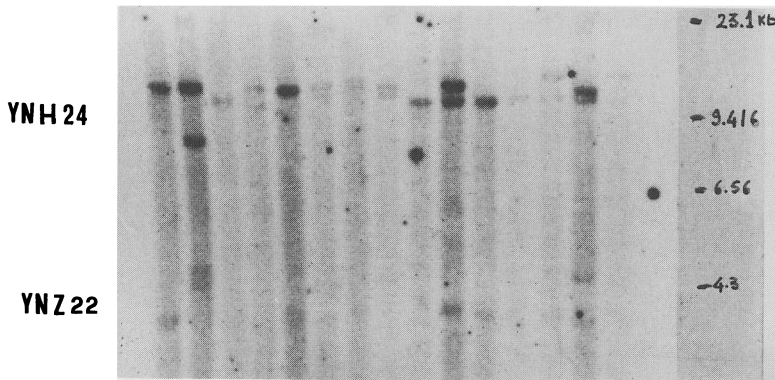


Fig.2. YNH24 and YNZ22 over a PstI filter blot (two overlapped autoradiographs have been photographed together)

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