

MINISATELLITES OF THE Y CHROMOSOME

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1. INTRODUCTION

In recent years, the use of VNTR polymorphisms has become one of the most successful changes in the field of forensic medicine for personal identification and paternity testing. VNTR sequences have been identified on all human chromosomes and usually they map at or close to the telomers (1). VNTR have been usually identified by screening human DNA cosmid or charomid libraries by multi locus probes, single locus probes at very low stringency conditions or by synthetic oligonucleotides (2,3,4).

The human Y chromosome, by contrast, shows a very low degree of polymorphism (5) with the exception of the pseudoautosomal region (4,6). VNTRs of this region show the same allelic pattern of autosomal VNTRs and are not linked to a specific sex. On the contrary, a Y-specific marker would be of great practical use for cases of sexual assaults or paternity testing when the alleged father is not available but male relatives are available.

Here we report the results of the screening of a Y-chromosome library and the identification of some minisatellite sequences of the Y chromosome.

2. MATERIALS AND METHODS

2.1 Library screening. About 800 ordered array cosmids, carrying most of the euchromatic region of the human Y chromosome (7), were replicated with a "hedgehog" onto Hybond-N filters and hybridized with the following probes: 33.15 (10), 33.6 (10), g3 (2), MS1 (2), MS43 (2), MS8 (2), 3'HVR (9), pMLJ14 (3), M13 (8). Hybridizations were carried out at 58°C in 7% SDS, 0,5 M phosphate buffer pH 7,2, 1 mM EDTA and 2 ug/ml sonicated and denatured *E. coli* CB767 DNA as competitor. The filters were washed at 58°C in 1xSSC and 1% SDS and exposed to X-Omat AR films.

2.2 Characterizing Y-specific patterns. The P32 labeled positive clones were used to probe Southern blots of four male and two female DNAs digested by following restriction enzymes: Hinf I, Bam HI, Eco RI, Mbo I, Sau3AI, Hind III, Pvu II, Hae III, Msp I, Alu I, Rsa I and Pst I. In this case the hybridizations and washes were carried out under high stringency conditions (2) without any competitor DNA.

2.3 Identification of minisatellite specific fragments.

Some positive clones, probed on human DNA, gave a "smear" and were subcloned. Each of these clones was digested with three restriction enzymes (Sau3AI, HindIII and PvuII), electrophoresed through a 1% agarose gel in TBE buffer, Southern blotted and hybridized with probes 33.15, 33.6, g3 and MS1. The cloning vector Lorist B (11) was used as negative control. The hybridization conditions were the same adopted for the screening of the library. Some of the minisatellite

fragments were recovered from the gel, subcloned in Bluescript and then used to probe human DNA.

3. RESULTS

We identified six positive clones (Tab. 1): three clones were positive for probe 33.6, two clones were positive for probe 33.15 and one clone was detected by g3. The six positive cosmids were hybridized to male and female human DNA and cosmids #2.25 38, #2.2 89 and #2.2 78 gave specific monomorphic pattern. The Y chromosome specific bands were obtained when the male DNA was digested with HaeIII, Sau3AI (Fig. 1) but not when digesting with Hinf I. The clones #2.18 27, #2.23 70 and #2.2 19 gave a "smear" and were digested and screened with 33.15, 33.6, g3 and MS1. The fragments obtained from each of the cosmid insert proved to be positive to more than one of the minisatellite probes (fig.2). Most probably the cosmid inserts were composed of different families of repeated sequences. Five of the fragments obtained from the cosmid inserts were used to probe male and female DNA.

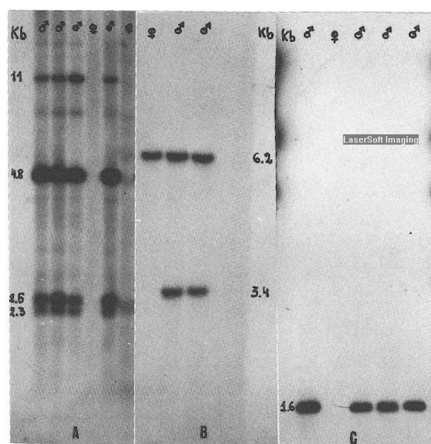


Fig.1 Y-specific monomorphisms detected by #2.25 38/Sau3AI (A), #2.2 89/HaeIII(B) and #2.2 78/HaeIII (C) digests.

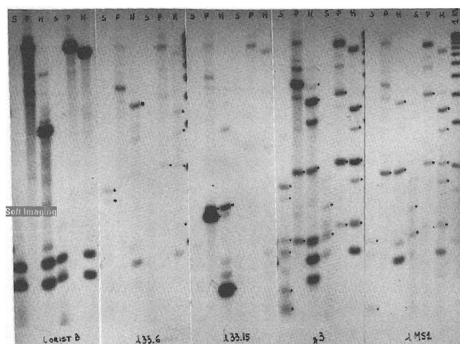


Fig. 2 Cosmids #2.23 70 and #2.2 19 digested by Sau3AI (S), PvuII (P) and HindIII (H) and hybridized with the probes shown below. Marked bands are minisatellite specific fragments.

The three positive cosmids and the 5 bands subcloned from the other three cosmids gave the following patterns:

- chromosome Y specific multi-band pattern: clone #2.25 38 and the 1.2 kb Pvu II fragment of clone #2.18 27
- chromosome Y specific single band plus one or more band in common with the X chromosome: clone 2.2 89 and the 2.1 kb Hind III fragment of clone #2.2 19.
- chromosome Y specific single band: clone #2.2 78; the 1.4 kb Hind III fragment of #2.18 27 and two Pvu II fragments (1.3 and 1.1 kb) of #2.23 70.

		Table 1	
PROBE	REFERENCE	POSITIVE CLONES	
33.15	(10)	#2.25 38 (A);	#2.2 89 (B)
33.6	(10)	#2.18 27 (C;A);	#2.23 70 (C;C); #2.2 19 (B)
g3	(2)	#2.2 78 (C)	
MS1	(2)		none
MS43	(2)		none
MS8	(2)		none
3'HVR	(9)		none
pMLJ14	(3)		none
M13	(8)		none

The letters in brackets refer to the pattern detected by the probe (see text for more details).

4. DISCUSSION

A Y specific cosmid library was screened with nine highly polymorphic probes. All positive cosmids gave invariably a monomorphic, although different, pattern. The screening of whole genome library failed in finding polymorphic minisatellites (12), possibly because the Y chromosome is only 1 % of the human genome. We were expecting that the screening of a selected Y chromosome library could lead to the identification of some polymorphic sequence of the chromosome but none of the 800 ordered array cosmid detected a polymorphism. This result can be explained by the absence of recombination events in this portion of the chromosome. Furthermore our results do not support the hypothesis (13) that others mechanisms as insertion, deletion, sister chromatid exchange, play a major role in generating new VNTR alleles, at least for this chromosome.

In conclusion, this work provides further evidence that the degree of polymorphism of the Y chromosome minisatellite sequences is very low or null.

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