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PCR DETECTION OF STRUCTURALLY ABNORMAL Y CHROMOSOMES

Shigeo NAGAFUCHI,[1] Satoko SEKI,[1] Yutaka NAKAHORI,[2] Takashi TAMURA,[1,3] Hironao NUMABE,[1,4] and Yasuo NAKAGOME[2,*]

[1]*Department of Congenital Abnormalities Research, National Children's Medical Research Center, Taishido, Setagaya-ku, Tokyo 154, Japan*
[2]*Department of Human Genetics, School of International Health, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan*
[3]*Department of Clinical Genetics, School of Health Sciences, Kyorin University, Miyashita-cho, Hachioji, Tokyo 192, Japan*
[4]*Department of Pediatrics, Tokyo Medical College, Shinjuku, Shinjuku-ku, Tokyo 160, Japan*

Summary Three probes each detecting a locus on the proximal long arm of the Y chromosome were partially sequenced. Thus, 3 sets of novel primers were developed which enable PCR detection of these 3 loci. Five previously reported primer sets, 3 on the short arm and each one on the centromere and the distal long arm, were mapped along with the novel three using a mapping panel consisted of 8 patients each with different structural abnormality of the Y chromosome. Now, PCR detection of these 8 loci covering an entire length of the Y chromosome has become possible enabling rapid screening of patients with Y chromosome aberrations.

Key Words PCR, STS, Y chromosome, deletion mapping

INTRODUCTION

Genetic linkage studies of the non-pseudoautosomal region of the Y chromosome are not possible because it does not form chiasma in meiosis. Therefore, deletion mapping is a necessary tool in the characterization of the human Y chromosome. Construction of a deletion map by Southern blotting using over ten different probes is laborious. However, the use of polymerase chain reaction (PCR) described by Saiki *et al.* (1988) should enable the analysis of many samples in a very short period of time.

We have developed 3 novel primer sets which detect 3 loci within the non-

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*To whom correspondence should be addressed.

fluorescent part of the long arm of the Y chromosome. As far as the authors are aware of, PCR detection of only one locus has been possible within this part of the Y chromosome (Lucotte *et al.*, 1991).

MATERIALS AND METHODS

Subcloning and sequencing of probes. Three probes were subcloned into the vector PUC18 and partially sequenced. The 3 probes detect 3 loci within a non-fluorescent part of the long arm of the Y chromosome. The probe 87-26 which detects locus DYS139 was digested with *Xba*I and a 1.65 kb fragment was subcloned into the *Xba*I site of the vector pUC18. The probe 87-19 (DYS132) was digested with *Hin*dIII and a 0.42 kb similarly subcloned in the *Hin*dIII site of pUC18. These 2 probes were originally described by Nakahori *et al.* (1991a) in which we estimated the size of the fragments slightly shorter. A 0.8 kb fragment was derived from the prove 49f (DYS1) by digestion with both *Eco*RI and *Xba*I. The fragment was subcloned into the pUC18 vector digested with these 2 enzymes. The 49f probe was originally described by Vergnaud *et al.* (1986) and a gift from Dr. J. Weissenbach.

Using a set of a universal and a reverse primer (Toyobo, Tokyo) spanning cloning sites of the vector pUC18, each of the 3 insert was partially sequenced by the dideoxy chain-termination method using the Sequenase Sequencing Kit (Toyobo). Out of about 170 bp sequences each representing either end of individual inserts, a 20–23 bp primer sequence was chosen. A set of two primers representing near both ends of an insert was designed to match in their length and as much as possible in their GC contents.

Using normal male and female DNA, PCR reactions were carried out using the 3 primer sets. The annealing temperature of each PCR reaction was set at 50°C and when a single male (Y)-specific band was not observed, a higher temperature was applied. When the use of a 65°C-annealing temperature was not successful in obtaining a distinct Y-specific band, different primer sequences were attempted.

Additional 5 loci were also examined by PCR reaction. Three loci were on the short arm, PABY, SRY, and AMGL. As to the PABY locus, a mixture of the PABA, PABB, and PABC primers simultaneously detects both X-specific (PABX) and Y-specific (PABY) sequences from the proximal border of the pseudo-autosomal region (Ellis *et al.*, 1990). The SRY primers have been described by Nakagome *et al.* (1991b) and are based on the sequences reported by Sinclair *et al.* (1990). The AMGL primers were designed from the sequences of the amelogenin gene (Nakahori *et al.*, 1991b), and the X-Y homologous primer sequences have been reported elsewhere (Nakahori *et al.*, 1991c). The DYZ3 locus close to the centromere was examined using Y1 and Y2 primers (Witt and Erickson, 1989). A primer set which detect the B segment of the DYZ1 locus was also used

Table 1. PCR primers.

Loci	Primers (References)	Primer sequences	Lengths (bp)	Temp. (°C)
PABY	PABA(1)	5′-GTACTACCTTTAGAAAACTAGTATTTTCCC-3′	Y=947	54
	PABB	5′-CTGCAGAAACAAGCTCATCAGCGTGACTAT-3′	X=771[b]	
	PABC	5′-GAATTCTTAACAGGACCCATTTAGGATTAA-3′		
SRY	SRY-1F(2)	5′-CAGTGTGAAACGGGAGAAAACAGT-3′	270	65
	SRY-2R	5′-CTTCCGACGAGGTCGATACTTATA-3′		
AMGL	AMXY-1F(3)	5′-CTGATGGTTGGCCTCAAGCCTGTG-3′	Y=788	65
	AMXY-2R	5′-TAAAGAGATTCATTAACTTGACTG-3′	X=977[b]	
DYZ3	Y1(4)	5′-ATGATAGAAACGGAAATATG-3′	170	55
	Y2	5′-AGTAGAATGCAAAGGGCTCC-3′		
DYS139	26ab-1F[a]	5′-ATTTCCTTGTCCAATTATCTTCT-3′	1,650	60
	26ab-2R	5′-AGTTCCATCCTTTATATAACTGT-3′		
DYS132	19a-1F[a]	5′-TAGGTGGTGGTGTTGGCTGA-3′	420	65
	19a-2R	5′-ACTCTGAGGTGCAGTTAGCC-3′		
DYS1	49fb-1F[a]	5′-AATAGAGCCTTATCAGCAGC-3′	820	65
	49fb-2R	5′-AGTCAGTCTGGATGTTTCAG-3′		
DYZ1	Y1-6F(5)	5′-AATTTGAGCATTCGTGTCCATTCT-3′	1,024	60
	Y1-4R	5′-AATGCCCTTGAATTAAATGGACT-3′		

[a] Present study. [b] The length of the X-specific band is different from the Y-specific band. A 2.5% gel was used for loci SRY, DYZ3, and DYS132. A 1.0% gel for all other loci. (1) Ellis *et al.*, 1990; (2) Nakagome *et al.*, 1991b; (3) Nakahori *et al.*, 1991c; (4) Witt and Erickson, 1989; (5) Nakagome *et al.*, 1991a.

(Nakagome *et al.*, 1991a). The locus represents a part of the repeating unit of the DYZ1 family on the distal long arm (Cooke, 1976; Nakahori *et al.*, 1986).

PCR procedures. A 50 μl of reaction mixture, containing 0.5 μg genomic DNA, 5 μl of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl pH 8.4, 15 mM $MgCl_2$, 200 μg/ml gelatin), 5 μl of 8 mM dNTPs, 5 μl each of 10 mM primers and 0.5 μl *Taq* polymerase, were applied to a thermalcycler PJ-1000 (Perkin Elmer Cetus). The reaction mixture was preheated to 94°C for 5 min before the PCR cycles. The cycles were routinely carried out 25 times, as follows: denaturation at 94°C for 1 min; annealing at the specific temperature listed in Table 1 for 2 min and extension at 72°C for 2 min. In the case of PABY, the cycle was carried out 30 times and annealing time was 1 min. Electrophoresis was performed on 1% or 2.5% agarose gel according to the size of the amplified sequences (see Table 1).

A mapping panel consisted of eight DNA samples from patients with structurally abnormal Y chromosomes were subjected to PCR amplification. They were cases 877, 1,027, 1,023, 534, 815, 1,018, 981, and 924. The results of Southern-blot analysis of these samples was previously reported (Nakahori *et al.*, 1991a). Normal male and female samples were amplified as controls. Genomic DNAs were prepared from peripheral blood leukocytes by the standard methods.

RESULTS

Three novel primer sets were established for the three loci within the non-fluorescent part of the long arm. Their sequences and annealing temperatures were shown in Table 1. As shown in Fig. 1a to 1c, each primer set, including that for the DYS1 locus, produces single distinct band specific to the male. The DYS1 locus has been known to show multiple bands with polymorphism when examined by the Southern-blot method using the 49f probe (Human Gene Mapping 11).

The presence or absence of amplified sequences by PCR reactions was in complete agreement with the results of Southern analysis described earlier (Nakahori *et al.*, 1991a). Results of PCR detection of the DYZ3 locus corresponded to the presence or absence of 50f2D locus in Southern blot analysis. The location and the order of the 8 loci detected by the present study are summarized in Fig. 2.

DISCUSSION

At least two genes, those coding azoospermy factor and growth control Y, are thought to be located on the long arm of the Y chromosome (Human Gene Mapping 11). The construction of a deletion map of the long arm would contribute to the search for these genes. Since deletions in clinical samples can be detected by PCR as well as by Southern analysis, efficient deletion mapping by PCR is possible at those loci for which sequence data is available.

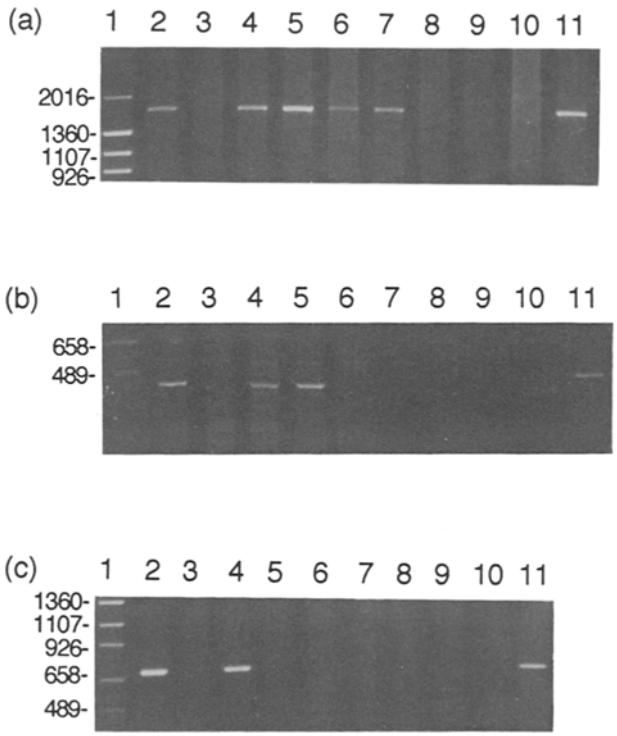

Fig. 1. Examples of the PCR products. Primer sets used were (a), DYS139; (b), DYS-132, and (c), DYS1, respectively. In lanes 1 to 11 are pHY marker (Takara; Tokyo, Japan), male control, female control, and clinical samples 877, 1,027, 1,023, 534, 815, 1,018, 981, and 924. Size markers are indicated on the left in bp.

We describe here three sets of novel oligonucleotide primers which detect three loci, DYS139, DYS132, and DYS1, in intervals 5 to 6 of the Y chromosome. Very recently, a set of primers for DYS1 has been developed based on the 49f-related 49a sequence (Lucotte *et al.*, 1991). An additional five sets of previously reported primers detect additional 5 loci (Ellis *et al.*, 1990; Nakagome *et al.*, 1991a, 1991b; Nakahori *et al.*, 1991c; Witt and Erickson, 1989). The loci detected by these eight sets of primers are distributed over the nearly entire length of the Y chromosome. Results of detailed deletion mapping are also available (Nakahori *et al.*, 1991a). There is an additional locus on Yp which is detectable by PCR (Tsukahara *et al.*, 1990). Unfortunately, the locus has been vaguely mapped on Yp using only the *in situ* technique.

These eight sets of primers can be used for rapid detection of structually abnormal Y chromosomes in clinical samples. Such is crucial in the screening of patients who are at high risk for gonadoblastoma. This tumor develops in patients

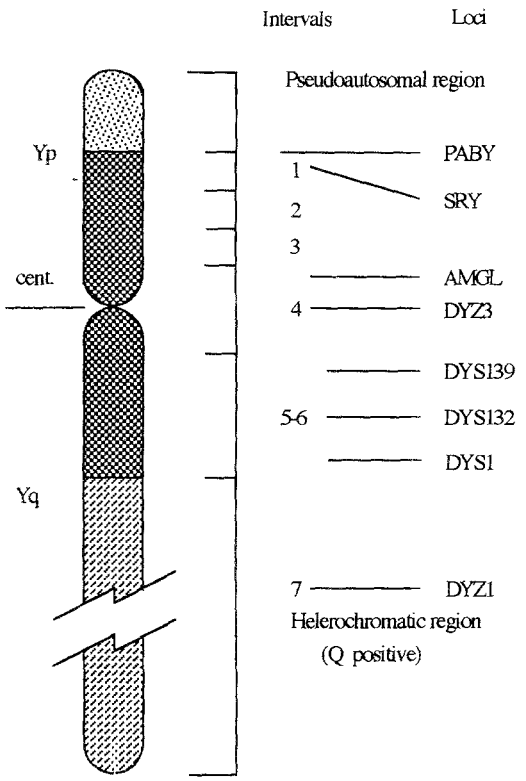

Fig. 2. The location and orders of the eight loci detected by PCR. Intervals 1–7 were defined by Vergnaud *et al.* (1986). The size of intervals are arbitrary.

with dysgenetic gonads who carry Y chromosome(s) or a part of it (Scully, 1970). Recently, we have shown that the development of gonadoblastoma is associated with a segment proximal to DYS132 (Nagafuchi *et al.*, in press). When such patients have a marker chromosome, PCR analysis can quickly determine whether the marker is of Y origin, and therefore if the patient is at risk for gonadoblastoma.

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