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VERY LOW RATE Y-CHROMOSOME MOSAICISM (1:5,400) DETECTABLE BY A NOVEL PROBE ENZYME COMBINATION

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Summary DYZ1 is a repetitive DNA family located on the long arm of the Y chromosome and is the major component of the Q-positive region. DYZ1 consists of about 3,000 copies of a 3.4 kb repeat unit which mainly consists of a tandem array of pentanucleotides, TTCCA. Because of this large number of repeats, DYZ1 has been used as a probe in Southern hybridization for sensitive and rapid detection of the Y chromosome. In cases of XX/XY mosaicism, however, autosomal sequences having homology to DYZ1 hinder the detection of the Y chromosome, especially when the ratio of the Y-bearing cells is low. To solve this problem and improve the detection limit, we have sought the optimum hybridization condition by changing several variables. These variables include the length of probes, the methods of probe labeling, the endonucleases used to digest the genomic DNA and the hybridization buffer. Here we show that the *Stu*I digestion of genomic DNA in combination with the nick translated DYZ1 probe significantly improves the detection limit of the Y-chromosome bearing cells. The presence of Y-chromosome bearing cells was detectable against a background of 5,400-fold female DNA.

Key Words Y-chromosome, DYZ1, low rate mosaicism

INTRODUCTION

The 3.4 kb Y-specific repeated DNA (DYZ1) was first described by Cooke (1976) as a band in the *Hae*III digested male genomic DNA. A complete repeat unit was cloned (pHY10) and nucleotide sequencing showed it to be a 3,564 bp fragment consisting of a tandem array of pentanucleotides, TTCCA (Nakahori *eI al.*, 1986). Although the exact length of the cloned fragment was 3,564 bp, here, we use "3.4 kb" to indicate the unit size of the repeated DNA family to be consistent

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with the previous study. Each unit is aligned tandem to a total of about 3,000 copies on the normal Y chromosome and forms a major component of the Q-positive region. Because of the high copy number, DYZ1 has been used as a sensitive and rapid probe to detect the presence of the Y chromosome both in *in situ* hybridization (Lau, 1985) and Southern hybridization studies (Morisaki *et al.*, 1988; Kobayashi *et al.*, 1988). However, the presence of autosomal sequences which hybridize to the probe has limited the detection of the Y-bearing cells to, at best, 1:500 against the background of female cells (our unpublished result).

Recently, the use of DNA probes derived from different regions of the Y chromosome have revealed the presence of Y-specific DNA in some 80% of 46,XX males (Müller *et al.*, 1986). Conversely, a small deletion of the short arm of the Y chromosome was described in a few females with gonadal dysgenesis. It appears that there is a critical segment on the short arm of the Y chromosome, which directs fetal gonads to testicular structure (Page *et al.*, 1987).

There are some seemingly paradoxical conditions. Cases of 46,XX true hermaphrodites and some 20% of cases of 46,XX male are among the examples in whom no Y-specific DNA signal has been detected by Southern hybridization studies (Waibel *et al.*, 1987). It is proposed that some of these cases may arise from undetectable mosaicism of Y-bearing cells (Iinuma *et al.*, 1975; Tantravahi *et al.*, 1986).

To address these problems, it is important to improve the detection limit of the Y-bearing cells. With this in mind, Stalvey and Erickson (1987) reported that the male specific fragment was detectable in a mixed (5% male and 95% female) DNA sample using probe Y97. In this report, we show that the presence of Y-chromosome material is detectable after a 5,400-fold dilution of male DNA with female DNA using a DYZ1 probe in combination with the use of endonuclease *Stu*I instead of either *Hae*III or *Eco*RI as in the standard technique.

MATERIALS AND METHODS

The genomic DNAs were isolated from lymphocytes of normal males and females. A series of male genomic DNA diluted with 4 μg of female genomic DNA was prepared. These serially diluted samples corresponded to mosaic levels of 1:200, 1:600, 1:1,800 and 1:5,400.

DNA was cut with the restriction enzymes according to the commercial suppliers instructions, electrophoresed and transferred to the nitrocellulose or nylon membrane as previously described (Maniatis *et al.*, 1982). Seven different enzymes were used including *Hae*III, *Eco*RI, *Stu*I, *Sau*3AI, *Dde*I, *Kpn*I and *Alu*I.

The DYZ1 hybridization probes were cut out of a pYN86002 plasmid which was carrying a pair of tandemly combined pHY10 fragments. Because of the tandem repeating nature of the pHY10 insert, we could cut out fragments of various length using the enzymes *Cla*I, *Eco*RI and *Bst*NI.

The probes were labeled with [^{32}P]dATP by three different methods which

included 3′ end labeling by Klenow enzyme, nick translation and multiprimer extension.

When using nitrocellulose membrane filter, hybridization was performed at 68°C in a buffer containing 6 × SSC, 0.1% SDS, 5 × Denhardt solution, 1 mM EDTA and 0.1 mg/ml salmon sperm DNA, with the filter being washed in 0.1 × SSC and 0.1% SDS at 68°C. When using nylon membrane filter, hybridization was performed in a buffer containing 6 × SSC, 1% SDS, 50% formamide, 5% dextran sulfate, 0.1 mg/ml salmon sperm DNA, and the filter was washed in 0.1 × SSC and 0.1% SDS at 60°C. Autoradiography was performed for 12 hr to 4 days at −80°C.

Several conditions were modified from the standard Southern hybridization procedure. Our standard conditions include using *Eco*RI for the genomic DNA digestion, nitrocellulose as a membrane filter, a 3.4 kb *Eco*RI fragment as the probe, and nick translation with [^{32}P]dATP for DNA labeling.

RESULTS

From looking at the serially diluted male genomic DNA samples we could ascertain certain requirements to obtain maximum detection with minimum background. There was no particular differences in detection when using probes of various lengths. The nucleotide length of the probe seemed to make no difference.

Labeling the DNA by either nick translation or 3′ end labeling gave similar, satisfactory results. When the probe was labeled by the multiprimer extension, the detection limit of the 3.4 kb Y-specific band was significantly decreased because of high background.

When the genomic DNA was digested with *Stu*I, we observed a distinct Y-specific band with low background. The other enzymes (*Sau*3AI, *Dde*I, *Kpn*I and *Alu*I) showed additional bands around the Y-specific band and the detection of the Y-chromosome bearing cells was disturbed.

While the nitrocellulose membrane filter gave good results, the nylon membrane filter gave a slihgtly better detection limit.

Figure 1 shows an autoradiograph obtained using the optimum conditions. It is seen that the lane containing 1: 5,400 male DNA gives a 3.4 kb Y-specific detectable band with no background.

DISCUSSION

We performed this study to develop a sensitive method for the detection of the presence of a small proportion of Y-bearing cells existing in a population of XX cells.

Since the DYZ1 family contains basically a tandem array of pentanucleotides (TTCCA) which is also a feature of human Satellite II and III DNA (Prosser *et al.*, 1986), the cross hybridization is inevitable. Though Cooke and McKay (1978)

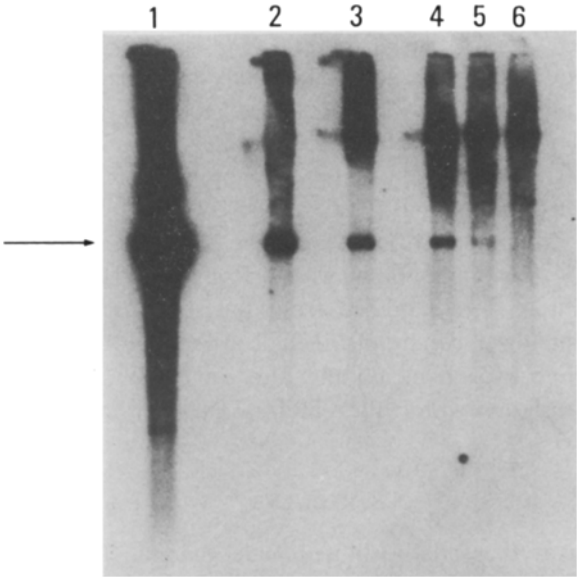

Fig. 1. Southern hybridization of male DNA serially diluted with female DNA using the DYZ1 probe labeled by nick translation. 3.4 kb band is indicated by an arrow. Lane 1, 1 μg male DNA; lane 2, 0.02 μg male / 4 μg female (1:200); lane 3, 0.007 μg male / 4 μg female (1:600); lane 4, 0.002 μg male / 4 μg female (1:1,800); lane 5, 0.0007 μg male / 4 μg female (1:5,400); lane 6, 4 μg female DNA.

described that the 3.4 kb periodicity of *Eco*RI and *Hae*III sites were specific to the DYZ1 family, there seems to be some autosomal sequences which cross hybridize to the DYZ1 probe and also have a periodicity similar to that of DYZ1 family (Fowler *et al.*, 1988 and our unpublished result). Therefore, when the genomic DNA was digested with *Eco*RI and *Hae*III, some ambiguity remains in the determination of the presence or absence of male DNA against the background of a large amount of female DNA.

In this study, we showed that the *Stu*I digestion of genomic DNA significantly improved the discrimination of the 3.4 kb Y-specific band. One explanation for this finding is that the cross hybridizing DNA of the autosomes lacks the *Stu*I site, and remains uncut as very large molecular weight material. This material being hard to Southern transfer to the membrane.

The reason that the hybridization buffer containing 50% formamide gave the best result may be that the formamide specifically interferes with the annealing of AT pairs and ultimately raised the stringency in such AT rich sequences.

We have been searching other methods for the detection of Y chromosome material including the newly described polymerase chain reaction (Saiki *et al.*, 1988). While it is easy to determine the sex in very diluted pure DNA samples

(Iwaya *et al.*, in preparation), the detection of the Y-bearing cells in the background of a large amount of XX cellular DNA is confusing. We believe the method we described here is the most reliable and reproducible among the techniques currently available.

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