Exonuclease digestion of human chromosomes for in situ hybridization and R-banding

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In situ hybridization of human chromosomes appears as one of the most attractive methods of gene mapping since it enables the locus being looked for to be directly visualized.

This technique however includes a critical step which is the denaturation of chromosomal DNA by different procedures such as heat, alkali or acid treatment (1). These affect the chromosome structure and decrease the good quality of the posthybridization chromosome banding which is essential to identify chromosomes and locate the probe.

Recently, Schmidt (2) reported that use of exonuclease III was a more gentle method to digest DNA on polytene chromosomes and to obtain a good chromosomal hybridization. This procedure has been applied on human chromosomes. The results are shown in figure 1. They showed the hybridization of the 3β-hydroxysteroid dehydrogenase (HSD β3) on human chromosomes. The cDNA coding for HSD β3 hybridizes on the p13 band of chromosome 1. This localization is the same as previously reported (3) with chromosomes denatured by alkali treatment. The data showed that exonuclease III is effective in digesting one of the two strands of human chromosomal DNA rendering the other strand available for in situ hybridization.

Moreover the chromosomal structure is much better preserved and R-banding that is used after the hybridization procedure is of high quality and obtained with more constancy. Figure 2 shows for example one chromosome 2 with R-banding after alkali treatment compared to exonuclease treatment.

Method

Chromosome preparations are obtained as previously described (4). The slides are treated with RNAse (100 μg/ml) for 60 min, washed in 2×SSC and 0.1×SSC at room temperature and digested with 50 units exonuclease III (Brl) in 10 μl exonuclease III buffer (5), 50 mM tris-Cl pH 8.0, 10 mM MgCl2, 1 mM mercaptoethanol, under a coverslip for 30—60 min, at room temperature. The exonuclease is washed out with 2×SSC, 2×5 min and the preparations are dehydrated through a series of ethanol and air dried. The hybridization and detection of DNA hybridized is carried out as previously described (6).

We can therefore confirm that exonuclease digestion is highly reliable to replace the denaturation step in 'in situ' hybridization procedures and that the identification of chromosomes is easier as chromosome bands are easily and constantly obtained.

REFERENCES


Figure 1. Five chromosomes 1 showing the hybridization of the HSD β3 cDNA probe after exonuclease treatment. The silver grains are found on the p13 band of chromosome 1.

Figure 2. R-banding of chromosome 2 after alkali treatment compared with exonuclease treatment. The R-bands obtained after exonuclease III treatment are sharper and the preservation of chromosomes is superior than with alkali treatment.