

DETECTION OF VARIATION IN THE RIBOSOMAL RNA GENE CLUSTERS BY A MODIFIED FLUORESCENCE *IN SITU* HYBRIDIZATION METHOD

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Summary Physical mapping of genes by fluorescence *in situ* hybridization (FISH) has become routine using fluorescein isothiocyanate (FITC) for probe detection and propidium iodide (PI) for chromosome staining. We have modified this conventional FISH method in a way that utilizes Texas red (TR) for signal detection and quinacrine mustard (QM) for chromosome banding. Using this Texas red and quinacrine (TRQ) method, we were able to identify individual acrocentric chromosomes with varying degrees of ribosomal RNA gene clusters. Two acrocentric chromosomes were found to carry extremely small number of rRNA gene copies as compared to the other eight counterparts in human diploid lymphoblastoid cell line GM00130B. Thus, the TRQ method allows one to probe for a specific sequence while identifying individual chromosomes and will be powerful for the chromosomal localization of various genes.

Key Words FISH, Texas red, Q-banding, rRNA gene, gene mapping

INTRODUCTION

Progress in the analysis of the complex human genome depends on mapping of a large number of DNA probes to individual chromosomes. The most advanced method for identifying the chromosomal locus of a human DNA segment is fluorescence *in situ* hybridization (FISH) (Bhatt and Mcgee, 1990). In the widely accepted FISH method, fluorescent signals of FITC are enhanced by counterstaining with PI and identification of individual chromosomes is not possible except R-banding is performed after probe hybridization (Lichter *et al.*, 1989; Viegas-Pequignot *et al.*, 1989; Cherif *et al.*, 1990; Takahashi *et al.*, 1991). At present, no method is available for concurrent chromosome identification and gene localization. Therefore, a rapid and simple technique for chromosome identification compatible with FISH is still required. We examined the possible use of a new combination of

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fluorescent dyes. Quinacrine (Q)-banding (Caspersson *et al.*, 1971) was used to identify chromosomes, while biotinylated probes were detected by TR-conjugated streptoavidin. By this combination (TRQ), we were able to determine the size of rRNA gene clusters on individual acrocentric chromosomes in human diploid lymphoblastoid cell line GM00130B. The TRQ method for FISH is simple and accurate and appears useful for physical mapping of human genes.

MATERIALS AND METHODS

Cell line and chromosome preparation. Human diploid lymphoblastoid cell line GM00130B was maintained as previously described (Minoshima *et al.*, 1990). The karyotype of this line appears normal and stable as indicated (Minoshima *et al.*, 1990). Metaphase chromosome spreads were prepared by the standard colcemid treatment, hypotonic shock and methanol/acetic acid fixation as described (Grill *et al.*, 1985). After RNase treatment, chromosomes were denatured in 70% formamide/2×SSC (1×SSC is 0.015 M sodium citrate/0.15 M sodium chloride, pH 7.0) for 3 min at 72°C, followed by dehydration in 70, 90, and 100% ice-cold ethanol (3 min each) and then air dried.

FISH and probe detection. A recombinant plasmid pHr14E3 containing 3' portion of 18S and 5' portion of 28S human rRNA gene including internal transcribable spacer (Ranzani *et al.*, 1984) was obtained from Japanese Cancer Research Resources Bank. The DNA was nick-translated at 16°C for 60 min in a reaction mixture containing 40 U/ml DNA polymerase I (BRL, Gaithersburg, Maryland), 68 ng/ml pancreas DNase I (Sigma, St Louis, Missouri) and biotinylated nucleotides (Biotin-7-dATP, BRL or Biotin-11-dCTP, ENZO, New York). Biotin-labeled probes were ethanol-precipitated, dissolved in a hybridization mixture (50% formamide/2×SSC/10% dextran sulfate, 1 mg/ml bovine serum albumin (BSA) (Boehringer, Mannheim, FRG) and hybridized as described (Lichter *et al.*, 1989). After post-hybridization washes and blocking with 5% nonfat dry milk (BRL) in phosphate buffered saline (PBS, pH 7.5), the probe was detected by means of FITC-conjugated avidin DN (FITC-AV, Vector Laboratories, Burlingame, California) or Texas red-conjugated streptoavidin (TR-SAV, Gibco BRL). The intensity of biotin-linked fluorescence was amplified by adding a layer of biotinylated goat anti-avidin antibody (Vector Lab.), followed by another layer of FITC-AV or TR-SAV.

Counterstain of chromosome. Chromosomes were counterstained with PI (5 µg/ml in PBS) for 3 min in the case of FITC-AV or with QM (Sigma, 10 µg/ml in MacIlvaine's buffer (pH 5.0)) for 20 min in the case of TR-SAV. The stained chromosome slides were washed without dyes for 30 min with vigorous shaking. We used low concentrations of QM (10 µg/ml) to avoid overstaining. Q-bands and Texas red fluorescence were stable in MacIlvaine's buffer (pH 5.0) for several days.

Laser scanning microscopy. The FITC, TR, QM, and PI were excited by an argon ion laser equipped on the MRC-600 system (Bio-Rad, Richmond, California).

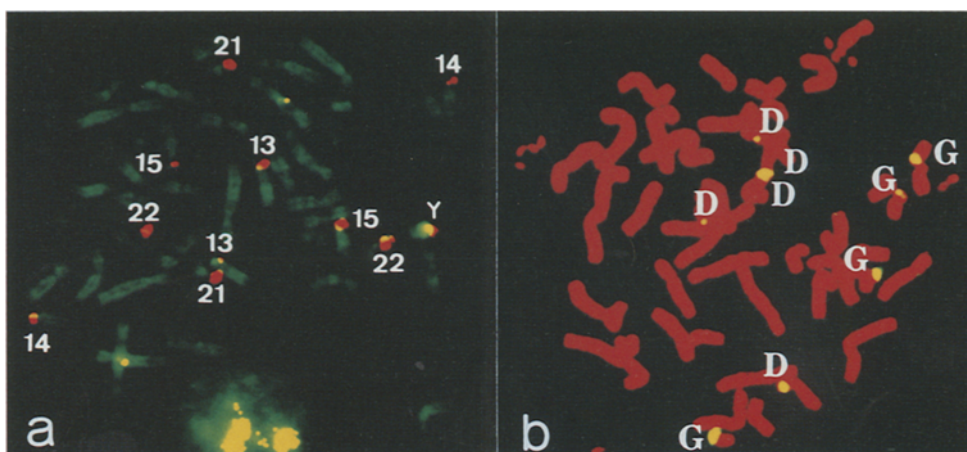


Fig. 1. (A) Identification of rRNA gene cluster regions by TRQ method with pHr14E3 as the probe. Numbers indicate D- and G-group chromosomes. (B) Detection of rRNA gene cluster regions by the conventional FISH method with pHr-14E3 as the probe. D, D-group chromosomes; G, G-group chromosomes.

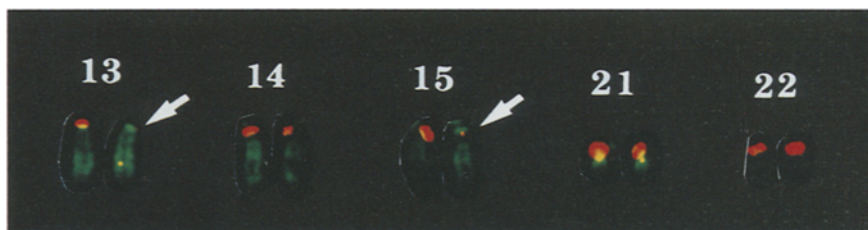


Fig. 2. Identification of rRNA gene cluster regions by TRQ method with pHr14E3 as the probe. Two chromosomes 13 and 15 exhibit the lowest signal intensities (arrows).

The fluorescence of FITC and QM, and fluorescence of TR and PI were detected by different photomultipliers after passing through specific emission filters (540 nm for FITC and QM, 600 nm for TR and PI, respectively). Thus, two separate images were obtained from the same slide and merged by computer graphics. They were photographed by MULTI SCAN (Toshiba, Tokyo).

RESULTS

We examined optimal conditions of QM counterstaining after detection of the hybridized probe by TR. This TRQ method was compared with a conventional FISH method which utilizes FITC and PI. The rRNA gene cluster was used as a model system for this comparison. The conventional FISH method yielded high levels of signal intensity on ten acrocentric chromosomes, but no banding information was obtained due to the homogeneous staining of chromosomes

(Fig. 1B). On the other hand, the TRQ method showed substantial fluorescent signals on the well banded chromosomes (Fig. 1A). Both methods revealed significant variations in the size of rRNA gene clusters among these chromosomes. The conventional FISH method revealed two D-group chromosomes as having the smallest rRNA gene clusters and the TRQ method identified these chromosomes as chromosomes 13 and 15 (Fig. 2, arrows).

DISCUSSION

We have successfully utilized a modified FISH method to rapidly recognize chromosomal banding pattern together with localization of fluorescent signals of DNA probes. Several FISH methods are available for the regional mapping of genes but these methods utilize post R-banding with or without Giemsa (Viegas-Pequignot *et al.*, 1989; Lichter *et al.*, 1989; Cherif *et al.*, 1990; Takahashi *et al.*, 1991) or pre-banding with Giemsa (Edwards *et al.*, 1990). G-banding requires relocation of specific metaphase spreads and two separate photographic steps. R-banding requires bromo-deoxyuridine treatment of cell cultures followed by UV irradiation and incubation with hot alkali (Therman, 1985). Q-banding used here has a number of advantages in its rapidity, easiness, and accuracy. The Q-banding has been successfully used for *in situ* hybridization in conjunction with radio-labeled probes (Shinkai *et al.*, 1989; Weremowicz *et al.*, 1990). Yet, radio-labeling is time consuming and requires statistical consideration. We used TR to detect signals of probes on the Q-banded chromosomes by the aid of laser scanning microscope and image analysis.

The copy number of rRNA gene is known to vary among chromosomes belonging to D- and G-groups (Ranzani *et al.*, 1984; Warburton and Henderson, 1979). In GM00130B cells, the conventional FISH method revealed that two D-group chromosomes carry much smaller numbers of rRNA genes than other acrocentric chromosomes (Fig. 1B). The TRQ method identified these chromosomes as one of homologs of chromosomes 13 and 15 (Figs. 1A and 2). The TRQ method clearly demonstrated the substantial variation of rRNA gene copy number among 10 acrocentric chromosomes. The TRQ method will be powerful for simple and rapid localization of various genes to their respective chromosomes.

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