

# A Simple, Rapid Technique for Precise Mapping of Multiple Sequences in Two Colors Using a Single Optical Filter Set

CAROL VILLNAVE JOHNSON,  
JOHN A. McNEIL,  
KENNETH C. CARTER, and  
JEANNE BENTLEY LAWRENCE

The development of fluorescence in situ hybridization technology makes possible the visualization of single-copy genes in metaphase and interphase cells, as well as of RNA transcripts in the nucleus and cytoplasm (reviewed by Lawrence et al. [1], McNeil et al. [2], Lichter et al. [3], and Gray et al. [4]). Here we provide details of current methodology for simultaneous detection and precise alignment of multiple nucleic acid sequences in two colors using standard microscopy and a single filter set. Included in this summary are specifications and characteristics of the Omega Optical dual-band filter for simultaneous visualization of Texas red and fluorescein fluorescence and a streamlined methodology for simultaneous detection of biotin- and digoxigenin-labeled probes. We describe a sensitive, fast, and efficient methodology whereby two or more probes are hybridized simultaneously and detected in just one step. Also indicated is our laboratory's experience concerning the relative sensitivity of biotin- and digoxigenin-labeled probes as well as results comparing the use of total human DNA and Cot-1 DNA as a competitor of repetitive sequence hybridization.

Detection of green and red fluorescence is routinely done using separate filter sets, and, importantly, the difference in optics between these filters results in a shift in the placement of the signals with respect to each other. This becomes particularly critical for applications where precise

placement is essential, as in gene mapping or detailed studies of nuclear or cellular organization. The use of a single filter set avoids the necessity for, and problems inherent in, double exposures or image processing and computerized superimposition. Omega Optical custom produces a dual-band filter consisting of a FITC-Texas red dichroic filter, a FITC-Texas red excitation filter with emission of light in the range of 484-502 nm and 578-593 nm, and a FITC-Texas red emission filter with emission in the range of 515-540 nm and 612-655 nm. This filter can be used to detect rhodamine in place of Texas red with very good results. Because the dual filter represents a compromise between the optimal wavelengths for Texas red and fluorescein, the signals observed are slightly weakened. However, this filter is used routinely in our laboratory with success both for abundant sequences and for single-copy sequences of 5-10 kb or more (see Figure 1). Applications for this type of filter have recently been reported in detection of chromosomal translocations [5] or mapping of single-copy sequences directly on fluorescence-banded chromosomes [6]. Use of the dual-band filter can allow precise localization of three clones on the chromosome or within the interphase nucleus [1-2], since, for example, a distinction between a red-green-red

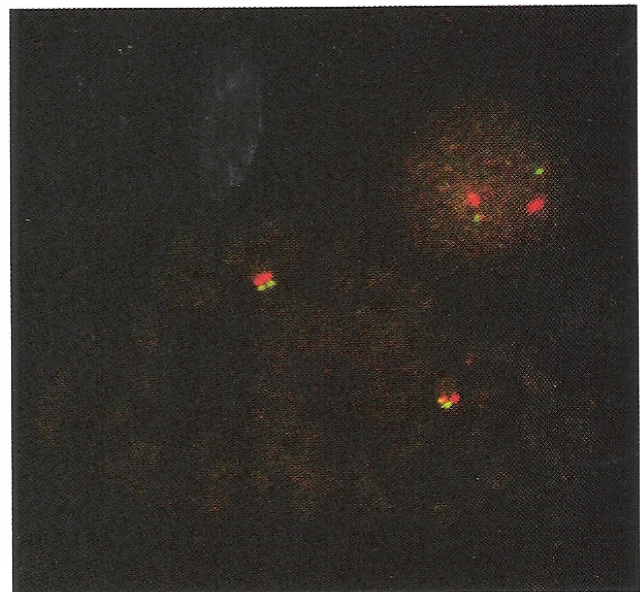


Figure 1. Simultaneous hybridization and detection of two DNA sequences in metaphase and interphase cells. Two cosmid clones on Chr. 17 (supplied by Peter O'Connell and Ray White) were labeled with either biotin or digoxigenin and detected with fluorescein-avidin (green) or rhodamine anti-digoxigenin antibody (red). This is a single photograph (without double exposure) taken with a single dual-band filter set using standard photomicroscopy.

From the Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts, USA.

Address correspondence to Dr. Jeanne B. Lawrence, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA.

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