

1

General Introduction to *In Situ* Hybridization

In situ hybridization techniques allow specific nucleic acid sequences to be detected in morphologically preserved chromosomes, cells or tissue sections. In combination with immunocytochemistry, *in situ* hybridization can relate microscopic topological information to gene activity at the DNA, mRNA, and protein level.

The technique was originally developed by Pardue and Gall (1969) and (independently) by John et al. (1969). At this time radioisotopes were the only labels available for nucleic acids, and autoradiography was the only means of detecting hybridized sequences. Furthermore, as molecular cloning was not possible in those days, *in situ* hybridization was restricted to those sequences that could be purified and isolated by conventional biochemical methods (e.g., mouse satellite DNA, viral DNA, ribosomal RNAs).

Molecular cloning of nucleic acids and improved radiolabeling techniques have changed this picture dramatically. For example, DNA sequences a few hundred base pairs long can be detected in metaphase chromosomes by autoradiography (Harper et al., 1981; Jhanwag et al., 1984; Rabin et al., 1984; Schroeder et al., 1984). Also radioactive *in situ* techniques can detect low copy number mRNA molecules in individual cells (Harper et al., 1986). Some years ago, chemically synthesized, radioactively labeled oligonucleotides began to be used, especially for *in situ* mRNA detection (Coghlan et al., 1985).

In spite of the high sensitivity and wide applicability of *in situ* hybridization techniques, their use has been limited to research laboratories. This is probably due to the problems associated with radioactive probes, such as the safety measures required, limited shelf life, and extensive time required for autoradiography. In addition, the scatter inherent in radioactive decay limits the spatial resolution of the technique.

However, preparing nucleic acid probes with a stable nonradioactive label removes the major obstacles which hinder the general application of *in situ* hybridization. Furthermore, it opens new opportunities for combining different labels in one experiment. The many sensitive antibody detection systems available for such probes further enhances the flexibility of this method. In this manual, therefore, we describe nonradioactive alternatives for *in situ* hybridization.

Direct and indirect methods

There are two types of nonradioactive hybridization methods: direct and indirect. In the direct method, the detectable molecule (reporter) is bound directly to the nucleic acid probe so that probe-target hybrids can be visualized under a microscope immediately after the hybridization reaction. For such methods it is essential that the probe-reporter bond survives the rather harsh hybridization and washing conditions. Perhaps more important, however, is, that the reporter molecule does not interfere with the hybridization reaction. The terminal fluorochrome labeling procedure of RNA probes developed by Bauman et al. (1980, 1984), and the direct enzyme labeling procedure of nucleic acids described by Renz and Kurz (1984) meet these criteria. Roche Applied Science has introduced several fluorochrome-labeled nucleotides that can be used for labeling and direct detection of DNA or RNA probes.

If antibodies against the reporter molecules are available, direct methods may also be converted to indirect immunochemical amplification methods (Bauman et al., 1981; Lansdorp et al., 1984; Pinkel et al., 1986).

Indirect procedures require the probe to contain a reporter molecule, introduced chemically or enzymatically, that can be detected by affinity cytochemistry. Again, the presence of the label should not interfere with the hybridization reaction or the stability of the resulting hybrid. The reporter molecule should, however, be accessible to antibodies. A number of such hapten modifications has been described (Langer et al., 1981; Leary et al., 1983; Landegent et al., 1984; Tchen et al., 1984; Hopman et al., 1986; Hopman et al., 1987; Shroyer and Nakane, 1983; Van Prooijen et al., 1982; Viscidi et al., 1986; Rudkin and Stollar, 1977; Raap et al., 1989). One of the most popular system is offered by Roche Applied Science: the Digoxigenin (DIG) System which is described in detail later in this chapter.

Many years ago, the chemical synthesis of oligonucleotides containing functional groups (e.g., primary aliphatic amines or sulfhydryl groups) was described. These can react with haptens, fluorochromes or enzymes to produce a stable probe which can be used for *in situ* hybridization experiments (Agrawal et al., 1986; Chollet and Kawashima, 1985; Haralambidis et al., 1987; Jablonski et al., 1986). Modified oligonucleotides can also be obtained with the DIG system (Mühlegger et al., 1990). Such oligonucleotide probes will undoubtedly be widely used as automated oligonucleotide synthesis makes them available to researchers not familiar with DNA recombinant technology.

This manual concentrates on two labeling systems:

- ▶ Indirect methods using digoxigenin (detected by specific antibodies) and biotin (detected by streptavidin)
- ▶ Direct methods using fluorescein or other fluorochromes directly coupled to the nucleotide

The ordering information in Chapter 6 lists all the kits and single reagents Roche Applied Science offers for nonradioactive labeling and detection.