Nucleic Acid In Situ Probing
Hybridization to Human Chromosomes of an Alkaline Phosphatase Labeled Centromeric Probe

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Abstract
The main principles that underly the use of nucleic acid probes for in situ hybridization are summarized. These include probe design, target preparation, hybridization formats and conditions, and signal generating systems. These principles underly the specific protocol that is described, namely the use of an alkaline phosphatase-labeled cloned sequence of the alphoid repeated DNA family as a centomere probe for human chromosomes.

Index Entries: In situ hybridization; centromere; chromosomes; nucleic acid probes.

1. Introduction
In the context of molecular biology, probes are molecules whose recognition capability allows for the detection of specific target molecules. Such detection may be accompanied by techniques and/or analyses that facilitate localization, quantification, and characterization of the target molecule. Immunoprobes exploit the specificity of the recognition capability of antibodies (probes) for antigens (targets). Nucleic acid probe use, on the other hand, is based on the recognition capability of one nucleic acid base sequence for a complementary sequence. Hybridization by hydrogen bonding between complementary purine (adenine, guanine) and pyrimidine (thymine or uracil, cytosine) bases of DNA and/or RNA is the mechanism for such recognition.

In situ nucleic acid probing (i.e., probing of the nucleic acid in a biological specimen affixed to a microscope slide) has benefited from the formulation of the principles that govern hybridization in other formats, such as filter and solution hybridization. Thus, this technology has matured rapidly and is an important tool in diverse molecular biology studies. These include gene mapping, identification of subsets of cell types and pathogens, gene expression research, and molecular pathology involving diagnosis and prognosis.

Nucleic acid in situ probing also has benefited from the merger of traditional immunocytochemical methods with traditional nucleic acid hybridization techniques, thereby extending the versatility and sensitivity of the method. Indeed, the applications often yield information not accessible by other methods.

The seminal aspects of nucleic acid in situ probing are as follows:

1. The target is DNA or RNA localized within a cell (in situ).
2. The recognition of target nucleic acid relies on base pairing (hybridization) of complementary bases of the nucleic acids and the formation of hydrogen bonds between the complementary bases.
3. The detection of the probe-target hybrid entails the visualization of a reporter molecule that is directly or indirectly attached to the probe.
The extension of this technology by virtue of adaptations from immunocytochemistry falls into three major categories:

1. Immunocytochemical reporter systems:
   a. Enzymatic (such as alkaline phosphatase and horseradish peroxidase).
   b. Fluorescent (such as fluorescein and rhodamine).
2. Biotinylated probes:
   a. Biotin–avidin or biotin–streptavidin complexes are used in direct affinity assays.
   b. Biotin–avidin or biotin–streptavidin complexes are used in indirect affinity assays.
   c. Biotin–avidin or biotin–streptavidin complexes are used in indirect immunoaffinity assays.
3. Antigen-complexed probes:
   a. Digoxigenin-labeled probes detected with anti-digoxigenin-fluorochrome conjugates.

These are illustrated in Fig. 1.

1.1. Probes and Hybridization Principles

Because the principles of base complementarity and hybridization underly nucleic acid probing, the hybridization partners—probe to target—may be DNA to DNA, DNA to RNA, RNA to DNA, and RNA to RNA. When DNA is serving as a probe or target, it must first be rendered single-stranded in order for hybridization to occur. RNA probes or targets are single-stranded; hence they do not require an initial denaturation. Nevertheless, some RNA molecules with a complex secondary structure may have to be relaxed in order to achieve best results.

The probes that have been successfully used vary in size from synthetic oligonucleotide...