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Perspective

Labeling and Nucleic Acid Detection Enzyme-Labeled Probes

Sakai Li*

Department of Laboratory Medicine, Saitama Medical University, Saitama, Japan

DESCRIPTION

Detection or purification-enabling tags may be added to nucleic acids. Other interacting molecules can be located or recovered using the generated nucleic acid probes. Nucleic acid probes frequently have radioactive phosphates, biotin, fluorophores and enzymes as labels. Additionally nucleic acids can be attached to other molecules or surfaces to enable targeted distribution or immobilisation using the same bioconjugation techniques used to create nucleic acid probes. There are various reagents available for quick and effective benchtop oligonucleotide labelling and they work well for producing certain amounts of probe or when numerous probes bearing the same label are required for mutational analysis. Enzymatic techniques provide a cost-effective way to label probes for small-scale probe generation needs. Chemical techniques allow for larger-scale reactions. There are chemical and enzymatic ways to make probes that are tagged at the 5′ or 3′ ends of the oligonucleotide as well as scattered throughout the sequence. The degree of labelling necessary and if the alteration will result in steric hindrance, which prohibits the intended interactions influence the approach to be used. Typically the high specific activity obtained from the haphazard insertion of label into a probe is advantageous for nucleic acid hybridization experiment. However, end-labeling is necessary in order to enable protein binding in experiments that call for protein interactions such as gel shift and pull-down assays.

Labeling and detection assays for nucleic acid

Depending on the labelling technique, enzyme-labeled probes are frequently detected using colorimetry while radioactive probes are best detected using autoradiography. When combined with colorimetric reactions, common probes such as digoxigenin and fluorescein-labeled probes can be utilized to facilitate multicolor probe identification (e.g. alkaline phosphatase). Similar to this most DNA polymerases allow for the incorporation of Biotin-16-dUTP during PCR (Polymerase Chain Reaction) as an additional labelling and detection technique. The sensitivity and resolution of the fluorescent microscope that the researcher has access to affects the success of fluorescent *in situ* hybridization which uses fluorescent probes to detect DNA sequences.

DNA and RNA applications with labels

Blotting is the process of transferring large molecules to solid-phase membranes identifies both DNA and RNA sequences in intricate combinations of nucleic acids by hybridizing the nucleic acid and the probe due to the specificity of labelled probes. Depending on the experiment, these techniques also enable the collection of valuable data such as analyses of gene expression, mRNA size and copy number frequently utilise *in situ* hybridization to find one or more probes with various labels. Fluorophores and haptens, which are referred to as Biotin and Digoxigenin in the latter case, are the most frequently employed labels for the creation of non-radioactive DNA or RNA hybridization probes. Fluorescence spectroscopy uses fluorescent probes to identify them immediately after inclusion. There are many fluorescent dyes available now with optical characteristics that span the whole UV-Vis spectrum and are compatible with standard industrial light sources and filter systems. However, there are significant differences in the dyes in terms of size/bulkiness, hydrophilicity and photostability and incorporation efficiency. Traditional dyes like rhodamine, fluorescein, and cyanine derivatives have been used the most frequently up to now but more recent years have seen a rise in use of new dyes. In contrast to fluorophores, biotin and digoxigenin are indirect labels because a second reporter molecule is needed to visualise them.

Correspondence to: Sakai Li, Department of Laboratory Medicine, Saitama Medical University, Saitama, Japan, E-mail: skaili123@gmail.com

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