

Digoxigenin Labeling

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Abstract

Digoxigenin is increasingly used as a label for nonradioactive detection of nucleic acids and proteins. A variety of methods for labeling are described as well as numerous applications of technology.

Index Entries: Digoxigenin; DNA; *in situ* hybridization.

1. Introduction

The use of digoxigenin as a label in both nucleic acid and protein detection has increased greatly in recent years. This increase has been due in part to the increased resistance to the use of radioactive detection in both the research and clinical laboratory. This pressure has forced us to look for other markers to use. Some of the other nonradioactive techniques that have been explored include direct enzyme coupling and streptavidin-biotin. The problem with direct enzyme coupling is the expense and the difficulty of producing a large number of different probes. Enzyme-coupled probes are best used in cases where a large number of hybridizations are carried out to screen for a single marker. Streptavidin-biotin is a comparatively simple system to use for labeling. However, its difficulties lie in endogenous production of the target, biotin. Digoxigenin does not have this drawback, since this molecule is only synthesized in digitalis plants, so endogenous production is not a problem in bacterial or mammalian systems. This steroid hapten can then easily be detected by using an antibody raised against it. This antibody can be coupled to an enzyme to provide for either chemiluminescent or colorimetric detection.

2. Methods

Labeling of DNA with digoxigenin can be done using one of four techniques. Each technique is

best suited to particular uses. End-labeling of DNA is particularly useful for making up mol-wt markers for Southern hybridization. Since the markers are made by digestion with endonucleases, they have an overhang that is very easily filled in. This technique is also well suited to labeling oligonucleotides.

End-labeling is performed using terminal transferase and digoxigenin-11-dUTP (1). Random hexanucleotide labeling is used to label large probes that are resistant to polymerase chain reaction (PCR). In random hexanucleotide labeling, the polymerase reaction is carried out using the Klenow fragment of DNA polymerase I (2). Nick translation is used to label probes that are resistant to both PCR and random priming. Nicks are made using DNase I, and then DNA polymerase I is used to fill in the nicks and insert the digoxigenin-11-dUTP. These probes are two to three times less sensitive than those made by the other methods (3). The method that is the easiest to use to produce large numbers of probes is the PCR. In this reaction, primers are designed to attach to the regions flanking the desired probe. The most common way of doing this is to synthesize primers that will bind to the multiple cloning site of the vector you have cloned the insert into. The digoxigenin-11-dUTP is then substituted for 5–10% of the dTTP in the reaction mix. It is best to use the minimum amount of the digoxigenin-11-dUTP that will provide a signal, since the large

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hapten molecule tends to decrease the efficiency of the PCR as concentration increases (4).

Oligonucleotides can be labeled using one of three reactions. The first reaction is 3'-end-labeling using terminal transferase in the same way it was used to label DNA. However, in this case, the marker molecule is digoxigenin-11-ddUTP. The drawback of this technique is that only one digoxigenin molecule can be added per oligonucleotide, since the reaction cannot continue past the dideoxynucleotide. This decreases the sensitivity of the probe. A more sensitive method of producing labeled oligonucleotides is to do 3'-tailing. In this reaction, terminal transferase is used to add a mixture of unlabeled nucleotides and digoxigenin-11-dUTP, producing a tail containing multiple digoxigenin residues. This technique, however, produces probes with higher background owing to the presence of the longer tail. These problems can be overcome by changes in the hybridization conditions. The final method of producing labeled oligonucleotides is hampered by the need to cap the 5'-end of the oligo with a specialized Aminolink residue. This method then allows the use of the same labeling molecule used to label proteins digoxigenin-3-*O*-succinyl-ε-amino-caproic acid-*N*-hydroxysuccinimide ester (digoxigenin-NHS). This molecule reacts with the free amino group on the Aminolink base to form a stable amide bond (5).

The labeling of RNA is done by *in vitro* transcription. This can be performed using one of three RNA polymerases (SP6, T7, T3). As in the case of DNA labeling, the label molecule is digoxigenin-11-dUTP. In order to make a labeled RNA probe, the DNA of interest must be cloned into the multiple cloning site of a plasmid vector adjacent to the RNA polymerase promoter site. This construct is then linearized with a restriction enzyme to produce a template of a set length. The use of RNA polymerase will then allow the creation of runoff transcripts of uniform length. One digoxigenin-11-dUTP is incorporated for every 20–25 nucleotides. Some procedures work better with shorter probes. To shorten probes produced by this method, alkaline hydrolysis can be carried out (6).

When producing cDNA libraries, it is possible to produce digoxigenin-labeled cDNAs. These labeled cDNAs are labeled by adding the digoxigenin-11-dUTP to the nucleotide mix in the cDNA procedure. The library is then amplified using AMV reverse transcriptase. These cDNAs can then be used just like any other DNA probes.

A final method that can be used to label both DNA and RNA is photodigoxigenin. Photodigoxigenin is a digoxigenin molecule linked via a hydrophilic spacer to an azido phenyl residue. When the nucleic acid and the photodigoxigenin are mixed and exposed to a 100-W mercury lamp, a reaction occurs in which a digoxigenin is incorporated in one of every 200–300 bases. This method produces probes with an approximate 100-fold decrease in activity. The detection limit in a dot blot is 10 pg of photolabeled material.

Proteins are labeled using a completely different digoxigenin molecule. Digoxigenin-NHS is reacted with the protein. Free amino groups on the protein react with the digoxigenin-NHS to form stable amide bonds. Nonreacted digoxigenin-NHS is then removed using a Sephadex® column (7).

3. Applications

Digoxigenin-labeled probes can be used in a variety of applications in both the research and clinical laboratory. **Table 1** shows the uses and sensitivity of probes produced in the reactions described above.

Primarily all of the applications that digoxigenin-labeled probes are used in are modifications of radioactive procedures. Digoxigenin procedures, however, do have some problems that differ from radioactive ones sufficiently to be worth describing. Many of the detection procedures, particularly in chemiluminescence, are based on using an antidigoxigenin antibody coupled to an alkaline phosphatase molecule. This alkaline phosphate breaks down substrates, such as AMPPD, and produces light. The difficulty arises in that alkaline phosphatases occur in human fingerprint oils. Because of this, any fingerprint on the blot will be detectable and cannot be stripped off. Another difficulty is that certain membrane types quench the chemiluminescent reaction and

Table 1
Sensitivity and Application of Digoxigenin Labeling Methods

Labeling method	Probe sensitivity	Application
End-labeling of DNA fragments	1 pg	Labeling DNA mol-wt markers
Random hexanucleotide labeling	0.1 pg	Northern blots, Southern blots library screening, dot/slot blots
Nick translation	0.5 pg	Northern blots, Southern blots library screening, dot/slot blots
PCR	0.2 pg	Colony hybridization, Northern blots, Southern blots, library screening, dot/slot blots
3'-End labeling of oligonucleotides	10 pg	<i>In situ</i> hybridization, Northern blots, Southern blots, library screening
3'-Tailing of oligonucleotides	1 pg	Northern blots, Southern blots, library screening
5'-End labeling of oligonucleotides	10 pg	<i>In situ</i> hybridization, dot/slot blots library screening, primer extension
RNA labeling by in vitro transcription	0.3 pg	<i>In situ</i> hybridization, Northern blots, Southern blots
cDNA libraries prepared with digoxigenin-11-dUTP	0.1 pg	Library screening, subtraction hybridization Northern blots, Southern blots
Labeling nucleic acids with photodigoxigenin	10 pg	Labeling DNA mol-wt markers Labeling RNA mol-wt markers
Protein labeling with digoxigenin-NHS	10 pg	Immunohisto/cytochemistry, Western blots, ELISA procedures

Table 2
Applications of Digoxigenin-Labeled Compounds

Molecule labeled	Hybridization technique	Target molecule	Source of target	Reference
Oligonucleotide	Slot/dot blotting	Chloramphenicol acetyl transferase mRNA	Bacteria	9
Papillomavirus genome	Dot-blot	HPV16 DNA	Genital lesions	10
Ribosomal DNA	<i>In situ</i> hybridization	Ribosomal gene mapping	Rice-field eel chromosomes	11
pR72h fragment	Colony hybridization	<i>Vibrio parahemolyticus</i> DNA	Seafood	12
Oligonucleotide	<i>In situ</i> hybridization	mRNA for human glial fibillary acidic protein and human S-100-Beta protein	Formalin-fixed, paraffin-embedded human and rat brain tissue sections	13
Human angiotensin- converting enzyme DNA sequence	Southern, dot-blot, Northern hybridization	Angiotensin- converting enzyme	Bovine aortic endothelial cells	14
<i>Escherichia coli</i> specific DNA fragment probes	Colony hybridization	Diarrheagenic <i>E. coli</i>	Stool samples	15

cannot be used. Only nylon membranes can be used since nitrocellulose and PVDF membranes have this quenching effect (8).

Digoxigenin-labeled probes have been used for everything from DNA fingerprinting to the detection of *Vibrio* contamination in seafood. All these procedures are based on hybridization. **Table 2** shows some of the variety of uses digoxigenin-labeled probes have been put to in recent years.

This table shows only a small portion of the usage of digoxigenin in recent years. Because of the ease of use of digoxigenin and its excellent sensitivity in a variety of detection systems, the use of digoxigenin should increase in coming years.

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