Topic Introduction

Digoxigenin

Michael R. Green and Joseph Sambrook

In molecular cloning, digoxigenin is used as a ligand that can be incorporated into DNA and RNA probes and detected after hybridization with an anti-digoxigenin-antibody enzyme conjugate. Methods to label nucleic acids with digoxigenin and to detect digoxigenin-labeled probes are introduced here.

INTRODUCTION

The cardenolide digoxigenin ($M_r = 390.53$) can be derived by chemical or enzymatic removal of four sugar residues from desacetylanatoside C isolated from *Digitalis purpurea* (Reichstein 1962) or it can be extracted directly from *Digitalis orientalis* and *Digitalis lanata* L. *Scrophulariaceae* (Maanich and Schneider 1941; Hegenauer 1971). The chemical structure of digoxigenin (Cardwell and Smith 1953, 1954; Pataki et al. 1953) is shown in Figure 1.

In molecular cloning, digoxigenin is used as a ligand that can be incorporated into DNA and RNA probes and detected after hybridization with an anti-digoxigenin-antibody enzyme conjugate (Kessler et al. 1989; Kessler 1991) (see Fig. 2). Digoxigenin-labeled probes can therefore be used in Southern, Northern, and dot-blot hybridizations. They also have considerable advantages for in situ hybridization, in part because the hapten digoxigenin is not present in animal cells (e.g., please see Tautz and Pfeifle 1989; Hemmati-Brivanlou et al. 1990). This absence eliminates background contributed by endogenous molecules. Such is not always the case with biotin-labeled probes. Digoxigenin-labeled nucleic acid probes can be stripped from nylon filters by heating the filters for 20–30 min to 37°C in a large volume of 0.1% SDS, 2 mM EDTA (pH 8.0) (Church and Kieffer-Higgins 1988). After washing, the filters can be reprobed without significant loss of efficiency or sensitivity.

Note that after several cycles of probing, stripping, and reprobing, the background of chemiluminescence may increase significantly because of the accumulation of alkaline phosphatase residue on the filter. This problem can be avoided by treating the filter with proteinase K and formamide between every second and third probing (Dubitsky et al. 1992).

LABELING NUCLEIC ACIDS WITH DIGOXIGENIN

For labeling of nucleic acids, digoxigenin is supplied by the manufacturer (Boehringer Mannheim) in two forms: digoxigenin-11-dUTP (DIG-11-dUTP; see Fig. 1) and digoxigenin-11-UTP (DIG-11-UTP). In each form, digoxigenin is coupled by an alkali-stable linkage and a spacer arm to deoxyuridine triphosphate and uridine triphosphate, respectively.

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

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Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top100784

FIGURE 1. Structure of digoxigenin-11-dUTP (DIG-11-dUTP). Digoxigenin is coupled by an alkali-stable linkage and a spacer arm to deoxyuridine triphosphate.

FIGURE 2. Detection of digoxigenin-labeled nucleic acid probes with BCIP/NBT or AMPPD.

TABLE 1. Methods of labeling nucleic acids with digoxigenin

Method of labeling	Enzyme	Number of digoxigenin molecules incorporated	Reference
Random priming	Klenow fragment	1 per 25–36 nt	Kessler et al. 1990
Nick translation	<i>E. coli</i> DNA polymerase I	1 per 25–36 nt	Höltke et al. 1990
Tailing	Terminal transferase	1 per 12 nt	Schmitz et al. 1991
Amplification by PCR	<i>Taq</i> and other thermostable polymerases	1 per 25 nt	Seibl et al. 1990
Transcription	T3, T7, and SP6 RNA polymerases	1 per 25–36 nt	Höltke and Kessler 1990
cDNA synthesis	Reverse transcriptase	1 per 25–36 nt	McCracken 1989

For review, see Kessler (1991).
PCR, polymerase chain reaction.

- DIG-11-dUTP is a substrate for *E. coli* DNA polymerase, the Klenow fragment of DNA polymerase I, thermostable polymerases such as *Taq*, reverse transcriptase, and terminal transferase. Digoxigenin can therefore be introduced into DNA by a variety of standard reactions including random priming, nick translation, amplification, end-filling, and 3'-tailing.
- DIG-11-UTP is incorporated into RNA during in vitro transcription of DNA templates by bacteriophage-encoded DNA-dependent RNA polymerases (T3, T7, and SP6 RNA polymerases) (please see Table 1).

Oligonucleotides that have been 5'-aminated during synthesis can be labeled with digoxigenin by reaction with NHS-digoxigenin (digoxigenin-3-O-methylcarbonyl- ϵ -aminocaproic acid-*N*-hydroxysuccinamide ester; Boehringer Mannheim) (Richterich and Church 1993).

DIGOXIGENIN-SPECIFIC ANTIBODIES COUPLED TO REPORTER ENZYMES

High-affinity digoxigenin-specific antibody is raised in sheep immunized with the digoxigenin-coated proteins edestin or bovine serum albumin. The most versatile of the immunological reagents available for detection of digoxigenin-labeled probes are Fab (fragment antigen-binding) fragments of antidigoxigenin immunoglobulin that have been coupled to alkaline phosphatase (Boehringer Mannheim). The best chromogenic substrates for immunolocalized alkaline phosphatase are the binary reagents 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and 4-nitroblue tetrazolium chloride (NBT).

Under optimal conditions, where high concentrations (50–100 ng/mL) of digoxigenin-labeled probe are used in the hybridization step and the color reactions are incubated for extended times (16–24 h), it is possible to detect <1 pg of target DNA in a dot-blot assay (e.g., please see Kerkhof 1992). However, far greater sensitivity and a more linear response are obtained with the soluble chemiluminescent substrate adamantyl 1,2-dioxetane phosphate (AMPPD; Life Technologies Corporation) (Kerkhof 1992; Bronstein et al. 1993). In AMPPD luminescent systems, which have gained rapidly in popularity in recent years, removal of the phosphate residue by alkaline phosphatase stimulates the substrate to emit chemiluminescence at 477 nm, which can be measured using a luminometer. The light occurs as a glow that persists for several hours in solution and for considerably longer on nylon surfaces (Tizard et al. 1990). Images of blots can be captured on X-ray film, on Polaroid instant black and white film (Kricka and Thorpe 1986), on a phosphorimager, or on a charge-coupled device (CCD) camera (Karger et al. 1993). At its best, chemiluminescent detection of digoxigenin-labeled probes is highly sensitive (0.03 pg of target DNA or RNA) and rapid (<30 min exposure). The method is therefore ~10-fold more sensitive and 50-fold faster than autoradiographic detection of ³²P-labeled nucleic acid probes. In addition, reprobing is simplified because stripping of the filter may not be required (Allefs et al. 1990). As a consequence of these advantages, chemiluminescent assays for alkaline phosphatase labels are used for detection of immobilized nucleic acid sequences in a wide variety of techniques involving blotting and DNA sequencing.

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Cold Spring Harb Protoc; doi: 10.1101/pdb.top100784 originally published online December 14, 2021

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