2. DIG Labeling

This section is designed to help you choose the labeling method that is best for your purposes. The section includes:

- A summary table showing the many labeling methods available, the amount of template they require, the sensitivity of the probes produced by the method, the applications for which the probes are suited, and the labeling kit or reagent that Roche Molecular Biochemicals offers for that method.

- A comparison of the three commonly used labeling methods: PCR labeling, random primed labeling, and RNA labeling. This table should help you choose between these three popular labeling methods.

2.1 Overview of Nonradioactive DIG Labeling Methods

<table>
<thead>
<tr>
<th>Method (Roche Kit Available)</th>
<th>Advantages of Method</th>
<th>Amount of Starting Template Required</th>
<th>Labeled Probe Can Detect</th>
<th>Labeled Probe May Be Used For</th>
<th>Comments</th>
<th>For Detailed Procedure, see Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Labeling (PCR DIG Probe Synthesis Kit, Cat. No. 1636090)</td>
<td>Requires only a small amount of template</td>
<td>Plasmid DNA, 10–100 pg (ideally, 10 pg)</td>
<td>Genomic DNA, 1–50 ng (ideally, use 10 ng)</td>
<td></td>
<td>Optimize PCR in the absence of DIG-dUTP before attempting labeling reaction</td>
<td>51 (Chapter 2)</td>
</tr>
</tbody>
</table>

Note: Scientists in our laboratories and others have developed techniques for optimizing the various DIG labeling methods. For information on optimization as well as detailed procedures for the labeling methods, see the pages indicated in the last column of the table.

Note: A comparison of the three commonly used labeling methods: PCR labeling, random primed labeling, and RNA labeling. This table should help you choose between these three popular labeling methods.
# DIG Basics

## DIG Labeling Overview of Nonradioactive DIG Labeling Methods

<table>
<thead>
<tr>
<th>Method (Reagents Available)</th>
<th>Advantages of Method</th>
<th>Amount of Start Up Template Required</th>
<th>Labeled Volumes Can (in µl)</th>
<th>Labeled Probe MAY Be Used For</th>
<th>Comments</th>
<th>For Detailed Procedure, see Page</th>
</tr>
</thead>
</table>
| Random Primed Labeling, High Efficiency (DIG-High Prime DNA Labeling and Detection Starter Kit I and II (Cat. No. 1 745 832) and II (Cat. No. 1 585 614)) | - Produces very sensitive probes  
- Reaction can be scaled up indefinitely | 300 pg DNA (for probes that can detect single copy genes)  
- Lower amounts of template may be used if labeling reaction is performed overnight | 0.10 – 0.03 pg DNA | - Genomic Southern blots  
- Library screening  
(Nanoworks in district blots and Northern blots) | Requires highly purified template  
- Very sensitive to template impurities  
- Resuspension purified template in H2O or Tris buffer. Do not use TE, because EDTA will inhibit labeling reaction. | 63 (Chapter 2)  
Note: For a comparison of this and other labeling methods, see page 13 of this chapter. |
| Nick Translation | - Allows control of probe length (important in in situ hybridization applications) | 1 µg DNA | Mainly used for in situ applications; sensitivity depends on target system | - In situ hybridization | Requires highly purified template  
- Template should not be denatured before it is labeled. |  — |
| Direct Chemical Labeling (DIG-Chem-Link Labeling and Detection Set, Cat. No. 1 038 602) | - Simple technique  
- Can label probes of all sizes  
- Reaction can be scaled up  
- Labels linear and supercoiled plasmid DNA, total RNA, and mRNA | 0.2–5.0 µg DNA/RNA | 0.10 – 0.03 pg DNA | - Labeling of total RNA, mRNA, or cDNA, e.g. for differential and array screening  
- Labeling of large amounts of DNA or RNA | Requires highly purified template  
- Allows direct labeling of mRNA for differential and array screening; no need to synthesize cDNA | 74 (Chapter 2) |
| RNA Labeling (DIG Northern Starter Kit, Cat. No. 2 039 072) | - Generates large amounts of probe  
- Label probe is completely free of vector sequences  
- RNA probes are single-stranded  
- RNA probes are more sensitive than DNA probes for analyzing Northern blots  
- DIG-labeled RNA probes can easily be fragmented for in situ hybridization | Performed DNA, linearized  
1 µg PCR product with promoter  
100–200 ng | 0.10–0.03 pg DNA  
0.10–0.03 pg RNA | - Northern blots  
- In situ hybridization  
- Library screening  
- Distinct blots | Requires highly purified template | 62 (Chapter 2)  
Note: For a comparison of this and other labeling methods, see page 13 of this chapter. |
| 3’ End Labeling (DIG Oligonucleotide Tailing Kit, Cat. No. 1 362 372) | - Requires only a small amount of template  
- Reaction can be scaled up indefinitely | 100 pmol oligonucleotide | 10 pg DNA | - Library screening  
- Distinct blots  
- In situ hybridization | Oligos should be purified by HPLC or EtOAc (Schleicher and Schuell)  
- Probes can be used without purification | 73 (Chapter 2) |
| 3’ Tailing (DIG Oligonucleotide Tailing Kit, Cat. No. 1 417 213) | - Requires only a small amount of template  
- Produces more sensitive probes than end labeling  
- Reaction can be scaled up indefinitely | 100 pmol oligonucleotide | 1 pg DNA | - Library screening  
- Distinct blots  
- In situ hybridization | Oligos should be purified by HPLC or EtOAc (Schleicher and Schuell)  
- Probes can be used without purification | 73 (Chapter 2) |
2.2 Comparison of PCR Labeling, Random Primed Labeling, and RNA Labeling

This section gives a brief overview of the similarities and differences between PCR labeling, random primed labeling, and RNA labeling methods. The information in this section should help you choose between these three popular methods for adding a DIG label to a hybridization probe.

How the Three Methods Work

2.2.1 A Closer Look at PCR Labeling

In PCR, a thermostable polymerase (e.g., Taq polymerase) can quickly amplify a specific DNA target over a millionfold. If the reaction mixture contains DIG-dUTP, the amplified DNA becomes a highly labeled, very specific, and very sensitive hybridization probe. The specificity of the PCR labeling reaction makes this technique especially suitable for labeling very short target sequences. PCR-labeled probes are especially suitable for single copy sequence detection on genomic Southern blots and rare mRNAs on Northern blots. Of course, they also work for library screening, dot/slot blots, and in situ hybridizations.

PCR labeling produces a very high yield of labeled probe from very little template. The minimum and maximum amount of template for PCR labeling ranges from 10 to 100 pg plasmid and from 1 to 50 ng genomic DNA. However, our experience indicates that using only 10 pg plasmid or 10 ng genomic DNA leads to optimal results. In general, cloned plasmid inserts give better results than genomic DNA. The sequence of the PCR primers determines what region will be amplified and labeled. That means the quality of the template preparation does not normally influence the PCR labeling reaction. Even very crudely purified plasmid preparations (e.g., prepared by boiling) may be used. It also means that PCR labeling requires less optimization than other labeling methods.
2.2.2 A Closer Look at Random Primed Labeling

Random primed labeling can label templates of almost any length.

Note: For very short sequences, use the PCR labeling method (Section 2.2.1 above) for best results.

In random primed labeling, Klenow enzyme copies DNA template in the presence of hexameric primers and alkali-labile DIG-dUTP. On average, the enzyme inserts one DIG moiety in every stretch of 20-25 nucleotides. The resulting labeled product is a homogeneously labeled, very sensitive hybridization probe (able to detect as little as 0.10 – 0.03 pg target DNA).

Note: The spacing of the DIG molecules is very important. If DIG molecules were closer to each other, steric hindrance would prevent the large anti-DIG antibody from recognizing and binding the labeled probe.

These labeled probes are especially suitable for single copy gene detection on genomic Southern blots and in screens of recombinant libraries. Of course, they also work for dot/slot blots, and Northern blots.

Since each primer has a different six-base sequence, the labeled probe product will actually be a collection of fragments of variable length. Thus, the labeled probe will appear as a smear, rather than a unique band on a gel. The size distribution of the labeled probe depends on the length of the original template.

Example: Labeled fragments generated from a 2 kb template will range from approximately 300–1500 bp with most being approximately 800 bp long.

Unlike PCR labeling, random primed labeling requires highly purified templates [preferably prepared by the High Pure Plasmid Isolation Kit (Cat. No. 1 754 777 or 1 754 785) or density gradient (CsCl) centrifugation].

2.2.3 A Closer Look at RNA Labeling

Labeled RNA probes are generated by in vitro transcription from a linearized DNA template. During the transcription, many RNA copies of the DNA product are made and each is labeled with DIG-UTP. The yield of the probe from the RNA labeling reaction is very high. The labeled sequence is unique and highly labeled.

The unique template sequence must be downstream from a viral promoter (e.g. for T7, SP6, or T3 RNA polymerase).

The template may be either highly purified, linearized plasmid DNA or a PCR product which has had a suitable promoter added during amplification.

Labeled RNA probes are very sensitive. In fact, DIG-labeled RNA probes offer much better sensitivity than DIG-labeled DNA probes for detecting RNA targets. Thus, RNA probes are especially suitable for detection of rare mRNAs on Northern blots. Of course, they also work for Southern blots, library screening, dot/slot blots, and in situ hybridizations.
**2.2.4 Summary Table: Comparison of Important Parameters for the Three Labeling Methods**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCR Labeling</th>
<th>Random Primed Labeling</th>
<th>RNA Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of template required to produce probes that can detect single-copy genes</td>
<td>10 pg plasmid, or 10 ng genomic DNA</td>
<td>300 ng–1 µg genomic DNA or plasmid</td>
<td>1 µg linearized DNA, or 100–200 ng PCR product with promoter</td>
</tr>
<tr>
<td>Preferred template for best results</td>
<td>Cloned plasmid insert, unpurified (Even plasmids prepared by &quot;quick preparation&quot; methods (mini-preps, maxi-preps) or by simple boiling of cells may be used.)</td>
<td>Cloned plasmid insert, highly purified (Preferably, prepare plasmid with the High Pure Plasmid Isolation Kit, Cat. No. 1 754 777, or use density gradient centrifugation in the presence of cesium chloride.)</td>
<td>Specially prepared PCR product (must contain SP6, T3, or T7 RNA promoter), purified</td>
</tr>
<tr>
<td>Purity of template required</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Optimal ratio, DIG-dUTP:dTTP</td>
<td>1:3 (for probes &lt;1 kb long), or 1:6 (for probes 1–3 kb long), or 1:6 from 1:8 to 1:10 (for probes &gt;3 kb long)</td>
<td>1:3</td>
<td>1:3</td>
</tr>
<tr>
<td>Length of labeled product</td>
<td>Unique (dependent on primers)</td>
<td>Variable (dependent on original length of template)</td>
<td>Unique (dependent on promoter and termination sites)</td>
</tr>
<tr>
<td>Nature of labeled product</td>
<td>Many copies of a unique labeled DNA</td>
<td>Multiple labeled DNAs, transcribed from fragments of original template</td>
<td>Many copies of a unique labeled RNA</td>
</tr>
<tr>
<td>Sensitivity of labeled probe</td>
<td>0.10–0.03 pg DNA</td>
<td>0.10–0.03 pg DNA</td>
<td>0.1–0.03 pg RNA or DNA</td>
</tr>
</tbody>
</table>

**Conclusions:**
The most flexible and powerful way to add DIG label to DNA is by PCR, particularly if highly purified template is not available. Random primed labeling will produce probes of equal sensitivity. DIG-labeled RNA gives the highest sensitivity for detection of target RNA.