9. Stripping and Reprobing the Membrane

After hybridization, the membrane can be stripped of DIG-labeled probe and rehybridized to a different probe. You can hybridize probes labeled with alkali-labile DIG (provided in most of our labeling kits) to a membrane, then strip the membrane with almost no loss of target sequences. The mild stripping procedures (described in the table below) allow multiple, sensitive reprobing experiments (Figure 8).

**Important Tip:** Membranes should never be allowed to dry before stripping. Once dried, the membrane cannot be stripped and reprobed.

**Note:** See Section 5, page 127 in Chapter 2 for step-by-step versions of these procedures.

### Brief Procedures for Stripping Alkali-Labile Probes from Blot Membranes

| For This Type of Blot | To Remove | Use This Procedure
|-----------------------|-----------|------------------|
| **Southern** | Chemiluminescent product and probe | 1. Rinse in H₂O, 1 min.  
2. Wash²,³ with 0.2 M NaOH/0.1% SDS, 2 x 15 min, 37°C.  
3. Rinse in 2x SSC, 5 min. Store in 2x SSC. |
| | Colored product (from NBT/BCIP reaction) and probe | 1. Incubate in dimethylformamide at 50°–60°C for 1 h or more, until color has been removed. (Solution may need to be changed several times.)  
2. Rinse in H₂O, 1 min.  
3. Wash²,³ with 0.2 M NaOH/0.1% SDS, 2 x 20 min, 37°C.  
4. Rinse in 2x SSC, 5 min. Store in 2x SSC. |
| **Northern** | Chemiluminescent or colored product and probe | 1. Incubate in 50% formamide (deionized)/5% SDS/50 mM Tris-HCl (pH 7.5), 2 x 60 min, 80°C, in a sealed bag.  
2. Rinse in 2x SSC, 5 min, RT. Store in 2x SSC. |

1 Membranes should never be allowed to dry before stripping. Once dried, the membrane cannot be stripped and reprobed.

2 Researchers have successfully used this technique to probe a single Southern up to 20 times. Do not use a higher concentration of NaOH (e.g. 0.4 M NaOH at 65°C, as listed in some published procedures). Never use this alkali stripping procedure for Northern blots.

3 Do not store the NaOH solution in glass containers. Use only plastic containers for storage.

4 An alternative stripping solution for RNA probes is 90% formamide/10 mM sodium phosphate (pH 7.5). Do not use NaOH, since strong alkali will destroy the target RNA.

5 Prepare all solutions fresh, using only RNase-free reagents.

6 This stripping procedure allows multiple stripping and reprobing reactions. We have been able to probe a single Northern blot up to 14 times (see Figure 8).
Stripping and Reprobing the Membrane

Stripping and Reprobing of Northern Blots

First round: Hybridization of a multiple-tissue Northern with DIG labeled actin probe and DIG labeled TSH receptor.

Second round: Hybridization with DIG labeled actin and DIG labeled TSH receptor.

2nd round: Hybridization with DIG labeled actin.

7th round: Hybridization with DIG labeled actin.

Figure 8. Stripping Alkali-labile DIG-labeled Probes from a Northern Blot for Multiple Reprobing Experiments. Two DIG-labeled RNA probes were used to analyze RNA from various tissues. One probe recognized actin RNA; the other recognized the TSH receptor (TSHr). The same membrane was hybridized repeatedly, either with both probes or the actin probe alone. Each probe was chemically detected using standard techniques (see Chapter 2). Between the experiments, the DIG-labeled probes were removed with 50% formamide/5% SDS/50 mM Tris (see table in Section 9 for details). The result from the first, second, sixth, and seventh round of hybridization are shown above.

Results: Each of the probes produced strong hybridization signals with no background. There was no substantial loss of target RNA during the stripping procedures. By using this stripping and reprobing technique, our laboratory was able to successfully analyze a single membrane up to 14 times.