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Strip Northern and Southern Blots

TR0028.0

Introduction

Following exposure of a Northern or Southern blot (either by classical isotopic methods or North2South® Chemiluminescent Technology), researchers often wish to strip off the first probe and detecting reagents so that they may reprobe for the same or different target. This Tech Tip describes several commonly used conditions for stripping probe from a Northern or Southern blot while maintaining the nucleic acid target on the membrane (Table 1).

Be aware that any stripping procedure has the potential to denature sensitive nucleic acids, rendering them unrecognizable by the probe. Should this occur, reprobing will not be possible, and a new blot will have to be prepared for each probing experiment. Furthermore, any physical defects of a membrane will be exaggerated when stripped and reprobed.

Table 1. Possible stripping conditions for Northern and Southern blots.

Stripping Solution	Strength	DNA	RNA	Temperature & Time
0.5% SDS	Mild	Yes	Yes	60°C for 60 minutes
5 mM Tris•HCl, pH 8.0, 2 mM EDTA, 0.1X Denhardt's Reagent	Mild	Yes	Yes	65°C for 2 hours
50% Formamide, 2X SSPE	Moderate	Yes	Yes	65°C for 1 hour
0.4 M NaOH, 0.1% (w/v) SDS*	Moderate	Yes	No	45°C for 30 minutes, then room temperature for 2 x 10 minutes
Boiling 0.1% SDS	Harsh	Yes	Yes	Pour on 100°C solution and let cool to room temperature; repeat 2 times
0.1X SSPE or SSC/0.5% SDS	Harsh	Yes	Yes	Pour on 100°C solution and let cool to room temperature; repeat 2 times

*Blots must be neutralized in TE after stripping.

Tips for Stripping and Reprobing Blots

- Wash blots with target side up
- Handle blots only at corners
- Keep blots wet between hybridization and stripping
- Keep blots wet after stripping
- Store blots in 0.1% SDS or 1X TE at 4°C
- Stripped blots may be dried and stored at room temperature for long-term storage. However, any probe remaining on the blot will become permanently bound once the blot is dried.

Test for Stripping Efficiency

For biotinylated probes detected using North2South® Chemiluminescent Hybridization and Detection Kit (Product No. 17095), test for stripping efficiency by re-applying streptavidin-HRP and add substrate as indicated in the protocol. If no signal is detected, then stripping was complete. If signal is detected, try a stronger stripping method.

For probes directly labeled with HRP and directly detected using North2South® Direct HRP Labeling and Detection Kit (Product No. 17195), add substrate as indicated in the protocol. If no signal is detected, then stripping was complete. If signal is detected, try a stronger stripping method.

Buffer Formulations

50 X Denhardt's Reagent
5 gm Ficoll
5 gm Polyvinylpyrrolidone
5 gm BSA
Adjust volume to 500 ml

20 X SSPE
800 ml H_2O
175.3 gm NaCl
27.6 g $NaH_2PO_4 \cdot H_2O$
pH to 7.4 with NaOH
Adjust volume to 1 L

20 X SSC
800 ml H2O
175.3 gm NaCl
88.2 g sodium citrate
pH to 7.0 with NaOH
Adjust volume to 1 L

TE, pH 7.4
10 mM Tris•HCl (pH 7.4)
1 mM EDTA (pH 8.0)

Related Pierce Products

77016 **Biodyne® B Nylon Membranes**, 25/pkg.

17095 **North2South® Chemiluminescent Hybridization and Detection Kit,** for use in detecting biotinylated probes

17195 **North2South® Direct HRP Labeling and Detection Kit,** for direct labeling of probes longer than 50 nucleotides and subsequent Northern or Southern blotting detection

89880 **Chemiluminescent Nucleic Acid Detection Module**

20148 **LightShift® Chemiluminescent EMSA Kit**