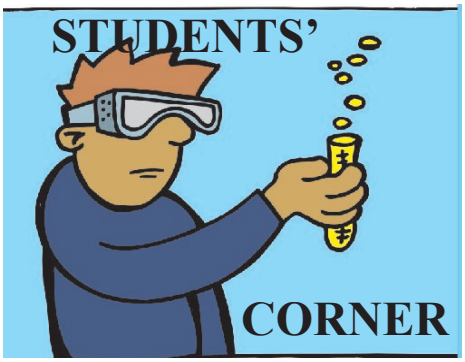

A METHOD FOR REMOVAL OF ANTIBODY PRECIPITATES FROM NON-RADIOACTIVE NORTHERN BLOTS.

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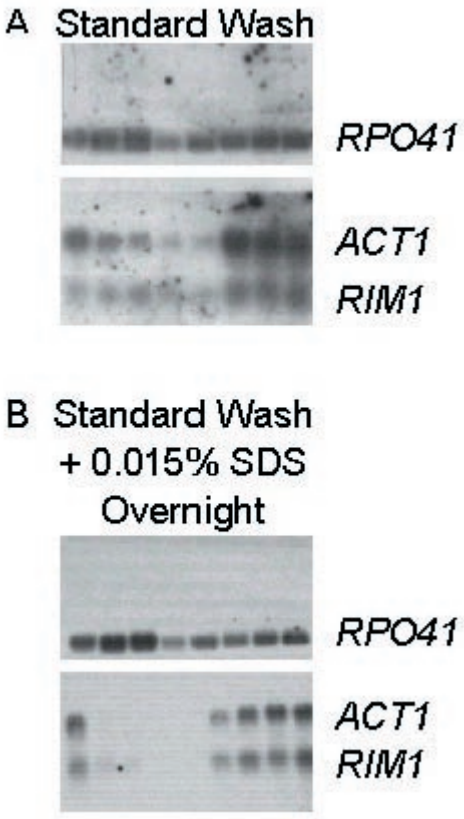
The use of non-radioactively labeled nucleic acids and enzyme-linked antibodies for probing blots of DNA, RNA and protein has significantly improved the ease and safety of these procedures. The sensitivity of these methods has been further increased with the development of chemiluminescent substrates. For example, digoxygenin (DIG)-labeled RNA probes hybridized to mRNA can be detected using anti-DIG antibodies coupled to alkaline phosphatase[2] (Roche Diagnostics). A strong signal is generated when the blot is subsequently treated with a chemiluminescent substrate, such as CDP-*Star*[2].

The use of enzyme-labeled antibodies introduces a step in which background signal can be obtained: incomplete removal of antibody or precipitation of aggregated antibody on the membrane can produce background signals that significantly interfere with visualization and quantitation of the desired hybridization signals (Fig. 1A). Strict adherence to manufacturers' instructions[2] prevents this problem in the vast majority of cases, but occasionally a blot with precipitated antibody results and information from valuable samples can be lost. We have developed a method that removes most of these precipitates (Fig. 1B). The blot is simply washed in washing buffer[2] (0.1 M maleic acid, 0.15 M NaCl, pH 7.5, (0.3% v/v) Tween 20), with the addition of sodium-dodecyl sulfate (SDS) to a final concentration of 0.015%. In some cases gentle shaking at room temperature for an hour is sufficient; however washing overnight is often required (Fig. 2B). This process almost completely removes the background signals, albeit with some loss of signal from the RNA-RNA hybridization and the resulting blot is suitable for quantitation and analysis.



This method is also applicable to Southern blots utilizing RNA or DNA probes.

In summary, following the manufacturer's instructions is critical for reproducibly obtaining high quality Northern blots. However, in the event that antibody precipitation occurs on the blot, further washing with SDS-containing buffer can remove most of the precipitate.

Figure Legend

Fig. 1 Northern blot on Hybond-N, using DIG-labeled RNA as probe. Total RNA was isolated from *Saccharomyces cerevisiae* as described[1], but scaled down tenfold. RNA obtained from approximately 1 OD 600/ml of cells was separated by electrophoresis on a 1.2% formaldehyde-containing agarose gel[3] and was blotted to Hybond-N (Amersham Pharmacia Biotech., Inc, Oakville, ON Canada), according to the manufacturer's protocol. Although Hybond-N is not the membrane recommended by Roche Diagnostics, excellent results can be obtained using it. The blots were hybridized to a mixture of DIG-UTP riboprobes against *RPO41, ACT1* and *ABF2*. Probe preparation, with the additional step of ethanol precipitation of the probe and hybridization were carried out as recommended[2]. Due to the rela-

tively high level of *ACT1* mRNA in the samples, this probe was diluted to approximately 0.75 ng/ml, while the *ABF2* and *RPO41* probes were utilized at the recommended concentration of 25 ng/ml in standard buffer containing 50% formamide. Detection of bound probe was performed using anti-DIG Fab fragments and the substrate CDP-*Star*, as described by the manufacturer. Signals were detected using Kodak X-OMAT film. **(A)** A blot with high background. A Northern blot was treated as described. However, high background, including sharp spots indicative of antibody precipitation[2] resulted, possibly due to inadequate centrifugation of the antibody solution prior to use. Exposure time was one minute. **(B)** The blot shown in (A) was washed overnight at room temperature with shaking in washing buffer that contained 0.015% SDS (w/v). Following this wash, the blot was rinsed briefly in ddH_20, followed by soaking for 2 min in detection buffer, re-application of CDP-*Star* and a 4-min exposure to X-ray film.

Acknowledgements
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3. Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.

Mingyi Li is currently a PhD candidate in Microbiology and the University of Manitoba. He is studying the regulation of genes involved in mitochondrial DNA replication in yeast. Mingyi came from China, where he first worked as a physician. Somehow he was inspired by scientific research and decided to start biochemical studies in medicine. Thereafter, he could not stop going forward in his studies from the animal level to the cellular level and now all the way to molecular level - which seems too far away from human level!

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ON A LIGHTER NOTE ...

From a recent test ...

In answer to "What is meant by 'selective advantage'?" ...

"Selective advantage is when one person chooses to mate with someone who they see as the most "fit" biologically, thus giving them an advantage to reproduce successfully."

This student is in training for a political career.

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