Autoclaving nitrocellulose membranes to improve detection of ubiquitin immunoreactivity.

1. Perform SDS-PAGE and transfer proteins to nitrocellulose according to your favorite protocol. (Do not use PVDF)

2. Rinse blot in distilled water for ~10 seconds.

3. Sandwich the blot between two sheets of filter paper and submerge as shown below.

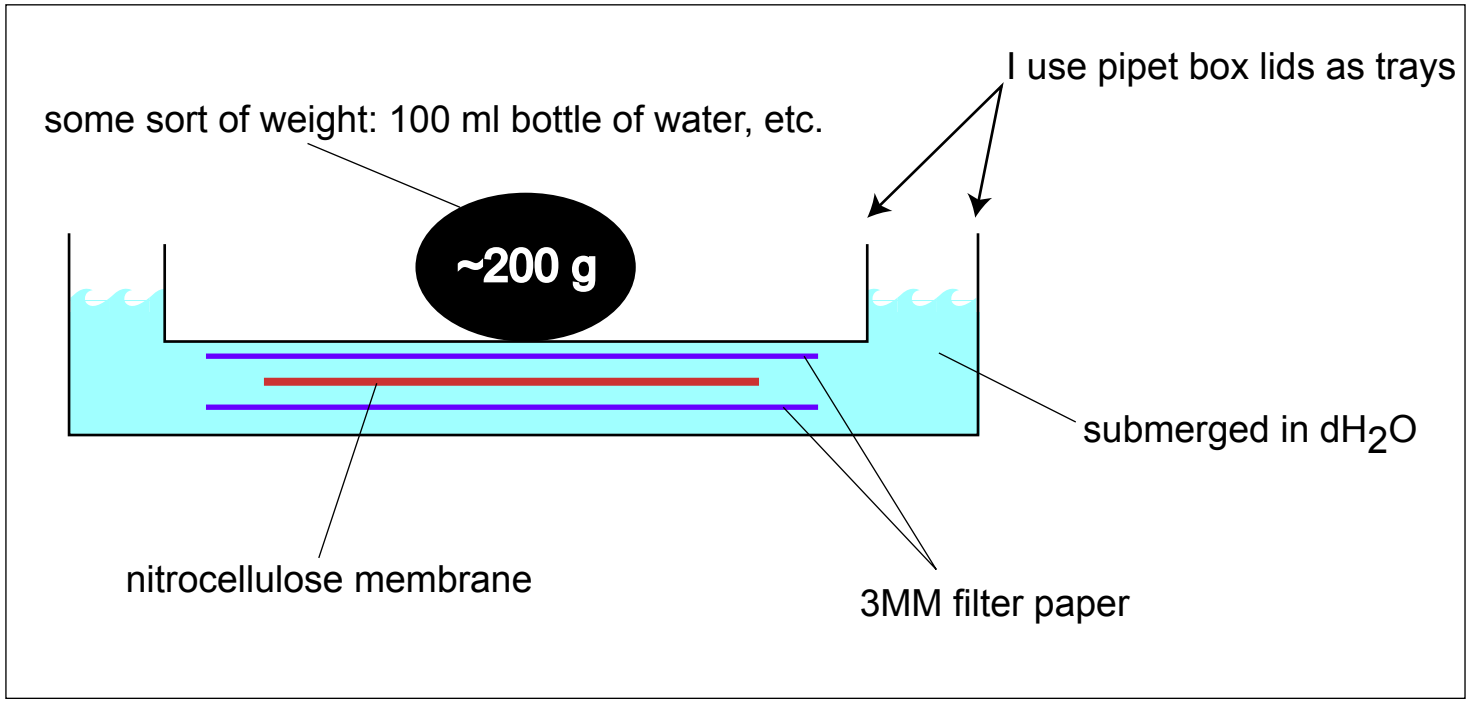

Figure 1. Sandwiching blot for autoclaving

4. Place in autoclave tray. **Autoclave 30 min. liquid cycle.**

5. Pour off excess water. The blot should remained sandwiched as in Figure 1 even though it is no longer submerged. Return sandwiched blot to autoclave. **Autoclave 15 min. dry cycle.**

6. Remove blot from from autoclave. Carefully disassemble blot after it has cooled. Use forceps and be careful not to tear the blot as you remove it from the filter paper.

7. Immunoblot with your favorite protocol, but see tips on the next page.

Some additional tips...

- I have observed that milk (and pure casein) is not the very best blocking agent for use with horseradish peroxidase and chemilluminescence. For instance, blocking with 20% heat-inactived bovine calf serum gave a four-fold higher signal than blocking with milk in otherwise identical experiments.

- My current block is Tris-buffered saline + 0.45% Tween 20 + 20% heat-inactivated (65°C for 30 min.) bovine calf serum.

- Many of the commercial antibodies against ubiquitin work very poorly in yeast. The anti-ubiquitin mAb from Zymed (http://www.zymed.com) works well.

The ability of autoclaving to increase sensitivity was described in
Swerdlow, P. S., Finley, D. & Varshavsky, A. "Enhancement of immunoblot sensitivity by heating of hydrated filters." *Anal. Biochem.* **156**, 147-153 (1986).

Nathan Bays, Hampton Lab, UCSD
nbays@ucsd.edu

http://hamptonlab.ucsd.edu