NORTHERN

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Citation: Weis, V. M., E. A. Verde and W. S. Reynolds. 2002. Characterization of a short form perdinin-chlorophyll-protein (PCP) cDNA and protein from the symbiotic dinoflagellate *Symbiodinium muscatinei* (Dinophyceae) from the sea anemone *Anthopleura elegantissima*. *J. Phycol.* 38: 157-163.

- Blot gel and UV crosslink membrane as described in blotting protocol.

- Preheat incubator and DIG Easy Hyb (about 15 ml for normal sized blot) containing 100 ug/ml salmon sperm DNA to 68°.

- Thoroughly wash and RNase Zap hybridization tube or use a 50 ml disposable concical tube.*

- Place membrane in tube, RNA side in (not towards the glass).

- Pour prehyb (DIG Easy Hyb + salmon sperm DNA) over membrane. Incubate, rotating at least 1 hr, 68°.

- Preheat 3-5 ml DIG Easy Hyb (NO salmon sperm DNA) to 68°,

- Iincubate probe briefly in almost boiling water or at 70° for a couple minutes to remove any secondary structure. Then place on ice briefly. Usually 1-2 ul of an RNA probe is a good amount. I usually add 8 ul RNase free water to the probe for the heating so that the couple ul does not evaporate away.

- Add probe to preheated DIG Easy Hyb.

- Pour off prehyb. Add probe solution.

- Incubate 68° overnight.

- Pour off probe solution.**

- Wash membrane with plenty (at least 25 ml) 2X SSC, 0.1% SDS ≥5 min, room temp. Repeat 1X. ***

- Wash membrane with plenty (at least 25 ml) preheated 0.2X SSC, 0.1% SDS 68° 20 min. Repeat 1X.

- Proceed with DIG detection protocol.

* You must be very careful using a 50 ml tube.
First, be sure it is the variety with a lid that actually fits easily into the standard hyb tubes. Second, using a red hot needle, burn a very small hole in the center of the lid so that pressure does not build in the tube.

Third, it must be inserted in the proper orientation in the hyb oven so that the rotation does not twist the lid off. The lid must be closest to the windows, or towards the left side of the oven. Fourth, put a rubber o-ring around the conical end to level out the tilt from the lid.

** You can supposedly save and reuse, but I prefer to use fresh probe each time if possible.

*** This should be done by hand rolling tubes on bench as we don't have an adequate alternative method. Using the shaker doesn't work well because tube bangs and coverage may be incomplete.