

SEMI-QUANTITATIVE PCR using Dig

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Citation: Weis, V. M. and W. S. Reynolds. 1999. Carbonic anhydrase expression and synthesis in the sea anemone *Anthopleura elegantissima* are enhanced by the presence of dinoflagellate symbionts. *Physiol. Zool.* 72(3): 307-316.

- First make cDNA in the usual manner using oligo(dT) and 2 ug total RNA.
- Use the following PCR mix, making up a sample number + 1 master mix of everything but template.

Per sample:

10X Buffer	5 ul
MgCl ₂ 25 mM	4 ul
dNTPs *	4 ul
Primer 1 50 uM	1 ul
Primer 2 50 uM	1 ul
Taq 5 u/ul	0.4 ul
cDNA	1 ul
H ₂ O	to 50 ul

dNTPs: 2 mM each dATP, dCTP, dGTP; 1.98 mM dTTP; 0.02 mM DIG-dUTP

To make: 6.0 ul each 20 mM dATP, dCTP, dGTP

5.94 ul 20 mM dTTP

1.20 ul 1 mM DIG-dUTP

34.86 ul H₂O

- After combining cDNA and master mix, mix thoroughly, then take 15 ul out of mix and put into each of two tubes. Now you should have three tubes per sample, two with 15 ul, and one with 20 ul. Label them somehow to differentiate.
- Use a program with normal temperatures and times for those primers. Remove one set of tubes after 17, 21, and 34 cycles. (You can vary the cycle numbers you want to do.)
- Run 5-7 ul of the 17 and 21 cycle samples on a 1-1.5% agarose gel. Blot onto a Boehringer Mannheim positively charged membrane. After blotting overnight, crosslink and detect as usual for DIG.
- Run the 34 cycle sample on a regular agarose gel with ethidium bromide and just check on UV light to see if reactions worked. These are too many cycles to be semi-quantitative.

Notes:

You must do a control with actin or some other housekeeping gene primers at least once with your cDNA to check for equal cDNA synthesis and equal PCRing among samples.

You can do the PCR without DIG-labeled dNTPs, substituting regular 2.0 mM dNTPs. Then after you blot the gel onto membrane, you would need to probe the membrane with a probe for the gene you amplified, i.e. hybridize, wash and detect. This is more work but leads to a prettier result.