Carbonate hydrolysis

1. The probe is hydrolyzed into fragments between 75 and 150 base pairs long so that they can efficiently penetrate the tissue sections during hybridization. To calculate the length of time to let the reaction go, use the following formula. I hydrolyze fragments longer than 450 base pairs to a final length of 0.2 kb (acceptable range is 0.1 kb to 0.2 kb).

$T(time) = (L_i - L_r)/K*L_i*L_f$

Where L_i is the initial length of probe, L_f is the final length of probe and K=0.11 kb/min.

2. Have RNA pellet resuspended in 100 ul of water. Add 60 ul 0.2 M Na_2CO_3 stock and 40 ul 0.2M $NaHCO_3$ stock (see below). Incubate for calculated length of time at 60C.

Stocks as follows:

200 mM $NaHCO_3$ (0.168g in DEPC water) (Checks grams as hydration state unknown)
200 mM Na_2CO_3 (0.212g in DEPC water)

3. Neutralize with 20 ul of 10% Acetic Acid (1 ml glacial + 9 mls DEPC water).

4. Precipitate probe with 21 ul of 3M NaOAC and 2 volumes of 100% ethanol and using 1 ul of 20 mg/ml oyster glycogen as carrier.

5. Incubate 2-3 hrs at -20C and then spin 30 min at 4C at max speed in the refrigerated centrifuge. Remove supernatant, wash pellet with cold 70% ethanol, remove all supernatant and air dry pellet.

6. Bring up in 100ul of 50% deionized formamide (4C fridge). Store at -20C indefinitely.

7. Quantify against DIG standard according to manufacturer's instructions. (1 day)