In Situ Hybridization to detect miRNA expression in *Xenopus* using Locked Nucleic Acid Probes

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1. Set Water bath at 20ºC below the Tm of the probe. (This is the only difference in the protocol we follow for in situ hybridizations using antisense RNA probe, except for step 4 of the LNA protocol—adding the probe).

2. 3' end labeling reaction from Roche (cat# 3 353 575)
    a. ice bucket
    b. __ of Exiqon LNA probe (100pmol) } 10µl total
    c. __of Nuclease Free H20
    d. 4µl reaction buffer (vial 1)
    e. 4µl CoCl2 (vial 2)
    f. 1µl DIG UTP (vial 3)
    g. 0.5µl terminal transferase (vial 4)
    h. incubate at 37ºC for 30 minutes
    i. place on ice and stop with 5µl of 0.1M EDTA (pH8)

3. Purify with G-25 Amersham (cat# 27-5325-01)
    a. Resuspend resin by vortex
    b. Loosen cap ¼ turn, snap off bottom
    c. Place column in a 1.5ml tube. Prespin for 1 min at 3K
    d. Apply sample to center. Spin 2min at 3K. Save this elution, it is your 3' end labeled LNA probe.

4. Remove prehybridization mix, discard, and replace with 600µl of hybridization mix containing 6µl of the LNA probe.

Example of finished product in a day 5 tadpole using gga-miR-124a probe: