RT-PCR from Zebrafish Explants

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For preparation of either 1 whole embryo or 1 explant (usually a combination of 3-5 individual animal caps or other explant tissue)

1) Transfer embryo/explant into eppindorf tube, remove as much embryo medium as possible without disturbing the sample
2) Add 100 µL of TRIZOL solution (Invitrogen) [At this point, the samples can be stored at –80°C or processed immediately.]
3) Mix the sample well by pipetting up and down. Add 20 µL chloroform and shake well, let stand at RT, 3 min.
4) Centrifuge at 12k X g for 15 min at 4°C
5) Recover the aqueous solution, avoiding the interface. To the aqueous phase, add 1 µL glycogen (20 mg/mL) and 50 µL isopropanol, let stand at RT, 10 min.
6) Centrifuge at 12k X g for 10 min. at 4°C
7) Resuspend pellet in: 5 µL 10X DNase Buffer
   1 µL RNase-free DNase
   44 µL H_2O
   Incubate at 37°C for 30 min. to 1 hr.
8) Extract the reaction 1 time with phenol:chloroform
9) Precipitate with: 1 µL glycogen (20 mg/mL)
   5 µL 3M NaOAc
   112.5 µL EtOH
10) Resuspend in: 11 µL H_2O for cap samples
    250 µL H_2O for whole embryo samples
11) RT reactions: 11 µL RNA (either the entire cap sample, or a small part of WE sample)
    1 µL 25 µM random hexamers
    incubate at 70C for 10 min., then snap freeze in icy water
    add: 4 µL 5X 1st strand buffer
    2 µL 0.1M DTT
    1 µL 10 mM dNTPs
    1 µL SuperScript Reverse Transcriptase (do NOT add for –RT samples)
    incubate at 37°C for 2-4 hr., then heat inactivate enzyme at 95°C for 10 min.
12) PCR reactions: 36.5 µL H_2O
    5 µL 10X buffer
    1 µL 10mM dNTPs
    0.5 µL HotStarTaq
    1 µL each primer (20 µM)
    5 µL template

    cycles: hot start (15 min. @ 94°C)
    1 min. @ 94°C
    1 min. @ 55°C
    1 min. @ 72°C
    ] X 40-45 cycles
    10 min. @ 72°C