RNA Isolation for qPCR

-Collect embryos or tissues in 1.5ml Eppendorf tube & remove excess media
-Add 10 volumes homogenization buffer (~250µl), triturate tissue to dissociate.
    Homogenization Buffer:
    50mM NaCL
    50mM Tris-Cl (pH 7.5)
    5mM EDTA (pH 8.0)
    0.5% SDS
    200µg/ml proteinase K (thaw on ice until ready to add to HB)
-Incubate homogenate for 1hr at 37°C
-Add an equal volume of phenol:chloroform, vortex vigorously for 1'
-Spin at 5,000g for 10' at RT
-Transfer the aqueous phase to a clean tube and repeat phenol:chloroform extraction
-Transfer aqueous phase to clean tube and add 0.1 volumes of 3M sodium acetate (pH 5.2) and mix well.
-Add 2.5 volumes ice cold 100% ethanol, then incubate on ice for 2hrs
-Centrifuge at 5000g for 15' at 4°C.
-Remove supernatant and allow pellet to dry at RT
-Resuspend pellet in 20-50µl DEPC H_2O, then add an equal volume of 8M LiCl.
-Incubate at -20° C for at least 3hrs to o/n.
-Centrifuge at 10,000g for 30' 4°C.
-Remove supe, then wash pellet with 70% ethanol, centrifuge 5' as above.
-Remove supe and air dry pellet.
-Resuspend nucleic acids in small volume of DEPC H_2O.
-Run 1µl of nucleic acid on 1% check gel.
-DNAse treat sample in 100µl volume
    Example: 14µl nucleic acid
    66µl DEPC H_2O
    10µl 10X DNAse Buffer
    10µl DNAse (Promega)
-Incubate for 2hrs @ 37°C
-Phenol/Chloroform extract then Ethanol precipitate.
-Resuspend RNA in a small volume of DEPC H_2O and spec.