Total RNA Isolation from Xenopus Embryos

(ref Maniatis Maual)

-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.