Ascidian RNA extraction

Last modified by R.W. Zeller on 24 February 2011

1) Grow embryos at 18ºC until desired time point. Pellet embryos in ~100 ul pellets.
2) Freeze at -80ºC for up to six months if desired.
3) To a 100 ul embryo pellet, add 300 ul of homogenization buffer:

	1 ml	**3 ml**	**6 ml**
1M Tris pH 7.5	**50 ul**	**150 ul**	**300 ul**
5M NaCl	**10 ul**	**30 ul**	**60 ul**
0.5M EDTA pH 8.0	**20 ul**	**60 ul**	**120 ul**
10% SDS	**50 ul**	**150 ul**	**300 ul**
DEPC H20	**to 1 ml**	**to 3 ml**	**to 6 ml**

4) Add 10 ul 20 mg/ml proteinase K
5) Grind in glass tissue homogenizer.
6) Transfer liquid to eppendorf tube on ice, rinse homogenizer with H20 and process additional samples if needed.
7) Phenol/Chloroform extract sample; phenol is saturated but unbuffered at a pH of about 6.
8) Transfer aqueous phase to new tube, add 300 ul homogenization buffer to phenol/chloroform and re-extract, combine aqueous layers.
9) Extract with equal volume chloroform.
10) Add 1/10 volume 3M NaOAC, pH 6, and 2.5 volumes of EtOH, ppt at -80ºC.
11) Store RNA at -80ºC until use.