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RNA EXTRACTION FROM CELLS

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PROCEDURE

1. Allow cells to dissolve completely in Solution D. *(Samples can be stored at -70ºC indefinitely)*
2. Add the following:
   a) 1/10 volume 2M Sodium Acetate pH 4. Mix completely by inversion.
   b) 1:1 volume DEPC H2O saturated Phenol. Mix completely by inversion.
   c) 1/5 volume Chloroform?Isomyl Alcohol mix (50:1). Mix completely by inversion.
3. Vortex for 10 seconds. Make sure entire solution is mixed. Solution will become white.
4. Chill on ice for 15 mins.

ALL REMAINING STEPS ARE AT 4ºC AND SAMPLES MUST BE KEPT ON ICE AT ALL TIMES.

5. Centrifuge at 14000 rpm for 20 min at 4ºC. (Should separate into 2 very different phases)
6. Remove aqueous phase (top) and transfer to clean tube, noting volume.
7. Add equal volume of ice-cold 2-propanol and allow overnight precipitation at -70ºC. *(Samples can remain here indefinitely*)
8. Centrifuge at 14000 rpm for 20 min at 4ºC.
9. Should see a white pellet.
10. Decant the supernatant. Be careful not to lose the pellet.
11. Wash pellet with ~600μl of 75% ethanol.
12. Centrifuge at 14000 rpm for 15 min at 4ºC.
13. Decant supernatant. Make sure to remove any traces of liquid.
14. Allow pellet to dry at room temperature. Pellet will become transparent. Do not over-dry the pellet.
15. Resuspend the pellet in 50μl of DEPC water. Allow the cells to sit on ice for at least half hour and then mix the solution by pipetting up and down.