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Isolating RNA is easy and fun.

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Abstract
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We have found that the common phenol or guanidium thiocyanate based procedures for isolating RNA simply do not repay the effort for the yields involved (0.2-0.3 mg total RNA per g fresh weight with guanidium salts). A number of observations led us to examine and slightly modify a procedure originally developed for tissue culture cells (Auffray and Rougeon, 1979, Eur. J. Biochem. 107:303). This somewhat unusual procedure is based upon lithium chloride/urea solubilization of the cellular contents and the ultimate precipitation of RNA at high ionic strength. The combination of effective solubilization, inhibition of RNAse activity, effective precipitation of RNA, and a minimum of handling results in higher yields (0.5-1 mg RNA per g fresh wt.) for much less effort.

Precautions

General precautions for handling RNA are applied. With the exception of the lithium chloride/urea solution - which is prepared fresh - all aqueous solutions are autoclaved before use. We are not enthusiastic about the use of diethyl pyrocarbonate (DEP) as a cure-all for RNAse problems and in any case it is not compatible with Tris buffers. The lithium chloride/urea solution can be neither autoclaved nor treated with DEP. The combination of chaotropism and high ionic strength seems to be completely effective in inhibiting RNAses.

Solutions

6M urea, 3M LiCl - freshly prepared, approx. 20 ml per g wet wt.
0.5% SDS, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA
10 M Ammonium acetate
Chloroform/isoamyl alcohol (24:1 v/v), Absolute ethanol
Liquid nitrogen

Method

1. Mycelium is harvested, washed, pressed dry and frozen in liquid nitrogen. Our typical growth conditions are an inoculum of 2x10^5 conidia m1^-1 in Vogel's medium with 2% sucrose as carbon + 0.2% Tween 80 incubated at 34° C for 24 h in a shaking incubator at 200 rpm.

2. Pulverize the frozen mycelium under liquid nitrogen in a mortar and pestle.

3. Resuspend the mycelium in 7-10 volumes of 6M urea, 3M LiCl. This may freeze, but this is unimportant, because it will thaw fairly quickly.

4. Pour the suspension, which should be fairly viscous, into the homogenizer vessel (50 ml vessel of the Sorvall omni-mixer) and wash the mortar out with an equal volume of urea/LiCl.

5. Homogenize the mycelium. In the 50 ml vessel of the Sorvall Omn-Mixer, we use 2 x 30-60 s on ice to homogenize effectively. Any conditions that produce a useful homogenate for other extractions should do.

6. Centrifuge the homogenate. We use 10,000 rpm (16,000 g max) in the Sorvall HB4 rotor for 20 min, 4° C. It is better to use a swinging bucket rotor because the resulting pellet is more compact and firmer. A clean separation between pellet end supernatant is very important. Ignore any lipid layer on the supernatant.

7. Decant the supernatant and store at -20° C overnight. This may freeze, but it does not seem to affect yields.

8. Centrifuge as before. Discard the supernatant and drain the pellet thoroughly.

9. Resuspend the pellet by vigorous vortexing with 5 ml 0.5% SDS, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. The suspension is often milky and viscous. If the pellet does not break up effectively, the suspension can be centrifuged (10,000 rpm, 16,000 g max 10 min 4° C, Sorvall HB4 rotor) and the pellet resuspended and extracted. This appears to give some improvement in the A260/A230 ratio (a measure of carbohydrate contamination).

10. Extract the suspension or pooled supernatants once with an equal volume of chloroform/isoamyl alcohol. It is important to obtain a good emulsion. Vortex vigorously 4 to 5 times for 15-30 s. Centrifuge (5000 rpm, 4000 g max, 10 min 4° C, Sorva11 HB4 rotor) to separate the phases.

11. Remove the aqueous phase, add ammonium acetate to 2.5 M and two volumes of ethanol to the aqueous phase. Precipitate the nucleic acids for 1 h at -70° C. Recover the precipitate by centrifugation (10,000 rpm, 20 min, 4° C, Sorvall HB4 rotor), wash the pellet once or twice with 70% ethanol and dry under vacuum.

12. Redissolve the pellet in the appropriate buffer for your next step.

Oligo (dT) cellulose chromatography

We have used protocols described by Schleif and Wensink (Practical Methods in Molecular Biology, Springer Verlag, 1981, pp. 168-170) and by Maniatis et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1982, pp. 197-198) to purify polyA^+ mRNA and find that they work equally well. It is important not to load more than 10 mg of total RNA per gram of oligo (dT) cellulose. If you find a poor yield of poly A^+ RNA (less than 1% of total RNA) it is possible that the column was overloaded. In that case we find that the non-binding (poly A-) fraction can be reapplied to a column after washing the column with alkali and buffer as described in the references cited above and more poly A+ RNA recovered from it. As judged by stimulation of in vitro protein synthesis and the pattern of translation products this second (and even third) extraction is as good as that obtained on the first pass. With reasonable yields of poly A^+ RNA (..20 ug at 4-5 ug ml^-1) we do not need to add carrier for ethanol precipitation. With smaller quantities we use Sigma tRNA, type V, and about 100 ug ml^-1 as carrier.

In general, we find that the RNA from step 12 has an A260/A280 of 2-2.1 and A26O/A230 of 2-2.2. The poly A+ content is about 1.5% of total. There is very little DNA as judged by agarose gel-electrophoresis. As judged by methyl mercury agarose gel electrophoresis, the RNA is up to 4000 nucleotides long with little evidence of degradation of the ribosomal RNAs. Using rabbit reticulocyte lysate supplied by Amersham for in vitro translation (Cat. No. N90, 0.2 ug poly A^+ in 2 ul double distilled H20 + 10 ul reticulocytes) we typically get a 40-fold stimulation of incorporation with polypeptides in excess of 200 kd being made. Depending upon the protocol used, we find that cDNA with median lengths between 700 and 1300 nucleotides is made.

A rider to be added is that we are isolating RNA from cultures growing under rather benign conditions. If cultures are grown under conditions likely to induce high levels of nucleases (e.g. phosphate or nitrogen limitation) then there may be problems with nucleases. In that case we stress the rapid freezing in liquid nitrogen and suggest homogenising the mycelium in a larger volume of urea/lithium chloride (say 25-40 vols) to dilute the nucleases.

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