Preparation of Total RNA from Aspergillus

Solutions:

A. 50mM Tris-HCl pH8.0, 0.3M NaCl, 5mM EGTA, 2% SDS

This protocol is designed for making RNA from a large number of samples beginning with 100-200 mg of wet weight material. It can be scaled up such that about 1 gram of wet weight of material is extracted in ~10 ml of buffer.

Harvest mycelia on Miracloth.

Place the mycelia into a microfuge tube and cap it with a cap which has had holes punched in it with a 16 gauge needle and freeze on dry iceor liquid nitrogen.

Lyophilize the frozen mycelia overnight. Note that the vacuum gauge should read <100 microns and preferably <50. It is essential that the vacuum is as good as possible because the RNA will be degraded if the samples thaw during drying or are not completely dried at the time of grinding.

Grind the freeze dried mycelia to a fine powder. Recently we have been using a small device which acts like a dounce homogenizer and fits a 1.5ml microfuge tube and is available from Kontes. They make two kinds a reusable Teflon and stainless steel version and a disposable plastic version. We have a Teflon and steel type and just rinse it with water and acetone between samples.

Transfer this material to a microfuge tube containing 500 ul buffer A and 250 ul of phenol at 65-70^{o}Cand immediately vortex at full speed for ~1 minute. At this point you can set the sample in a rack and get the remaining samples to this point. One should be careful not to try and extract too much material in a single go since you are likely to loose it to ribonucleases. Typically I use ~50ul powdered freeze dried mycelia and get 200-500ug of total RNA. Note that it is better to use a small amount of dried mycelia in a larger volume. This makes the extraction more efficient.

Add 250 ul of chloroform: isoamyl alcohol to each tube and vortex at full speed for ~1 minute.

Separate the phases by centrifugation in a microfuge for 5 minutes. Remove the aqueous phase and place it in a fresh tube.

Re-extract the aqueous phase with an equal volume of phenol: chloroform: isoamy alcohol 2X and then 2X with chloroform: isoamyl alcohol.

Precipitate both the DNA and RNA at -20^{o}C for 30 minutes or longer after adding 1ml ethanol.

Sediment the nucleic acids in the microfuge for 5 minutes wash once with 70% ethanol, dry and dissolve in 100-200 ul water.

The DNA hasn't appeared to be a problem when doing northerns but could be a problem for in vitro translations and PCR. So if you intend to translate your RNA you can selectively precipitate the RNA by the addition of an equal volume of 4M LiCl and incubation on ice for 3 hrs. Sediment the RNA precipitate in the microfuge for 5 minutes, wash it 2X in 70% ethanol, 50mM NaCl, dry it and dissolve in water. The RNA concentration must be at least 200 ug/ml in order to precipitate and you loose 5S and tRNAs.