

Isolation of total RNA from *Arabidopsis thaliana*

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The ability to isolate sufficient quantities of good quality RNA is very important for many types of experiments. Unfortunately, there are some inherent technical difficulties that sometimes cause the RNA to be degraded during the process of isolation or the yield to be too low. The first problem is that most tissues contain a considerable amount of ribonuclease (RNase) activity. A lot of the RNase present in the cells is sequestered in specific locations (e.g. the vacuole) in the living cell, where it will not normally degrade mRNAs that are present in the nucleus or the cytosol. However, when the cells are ground up, as they must be to extract the RNA, the RNases are let loose and can then start to degrade the RNA. Not only does the sample itself contain RNases, but so do the hands of the scientists doing the isolation, and residues of RNases can be present on scientific glassware, in water, on test tubes etc. It is very difficult to get rid of RNase because it is a very stable enzyme. Even autoclaving (which permanently denatures most enzymes) a solution containing RNase will not help. The RNase will be temporarily denatured by the heat, but upon cooling to room temperature it will just renature and become active again.

To do any kind of useful analysis with the RNA that is isolated, the RNA must be in the same state that it was in the cell. In order to prevent degradation of the RNA during isolation, a number of precautions must be taken. All reagents, glassware, tubes, pipet tips etc. that are used must be RNase free. All steps of the procedure must be done in a clean environment and clean gloves must be worn all the time. People who work with RNA regularly sometimes get a reputation for being extremely paranoid and obsessive about keeping things clean, but it is necessary to be that way if you want to get consistently good results.

Another problem is that many plant tissues contain a lot of polysaccharides (e.g. starch). Polysaccharides co-elute with RNA during many of the separation steps that are used to isolate RNA from other cellular components. As a result it is common with many RNA isolation procedures to end up with a big glob of polysaccharide mixed with a small amount of RNA.

There are a large number of published protocols for isolation of RNA from different tissues. All of them work well for at least some tissues of some organisms. There is unfortunately no universal RNA isolation protocol that works for all tissues of all organisms. Often trial and error is required to determine what protocol will work best to isolate a good yield of good RNA from the sample that you want to work with. Recently, a number of kits for isolation of RNA have become available. These kits can be very useful as they save a lot of time. We will purify RNA from our samples using the RNeasy Plant Mini Kit (Qiagen, catalog #74904).

EXPERIMENTAL PROCEDURE

Collect 200 mg samples of *Arabidopsis* tissue. Place each sample into a labeled 1.5 mL tube and immediately freeze it in liquid nitrogen. Store the samples in the freezer until you are ready to use them.

- 1.** Pre-cool a mortar and pestle with liquid nitrogen. Place an RNA sample into the mortar and grind it to a very fine powder. Do not allow the sample to thaw while you are grinding it.
- 2.** Transfer 100 mg of each sample to a 1.5 mL tube containing 450 μ L of Buffer RLT. Vortex vigorously. Allow the samples to incubate at room temperature for 3 minutes.
- 3.** Pipet the contents of your tube into the top of a “shredder” (purple) spin column, dude. Centrifuge for 2 minutes in a microcentrifuge at full speed. The RNA (and some other soluble stuff) will pass through into the collection tube.
- 4.** Dispose of the column and transfer the flow-through fraction from the collection tube to a new 1.5 mL tube. If there is a pellet in the collection tube, take care not to disturb it and transfer only the solution. Add 250 μ L (0.5 volumes) of ethanol to your solution in the new tube and mix well.
- 5.** Apply your solution to the top of an RNeasy spin column (pink). Centrifuge for 30 s in a microcentrifuge. The RNA will be bound to the membrane of the column at this point. Use a pipet to remove the flow-through fraction from the collection tube. Place the RNeasy column back onto the empty collection tube and discard the flow-through fraction.
- 6.** Add 700 μ L of Wash Buffer RW1 to the top of your RNeasy column. Centrifuge for 30 s in a microcentrifuge. Dispose of the flow-through fraction and place the column onto a new 2 mL microcentrifuge tube.
- 7.** Add 500 μ L of Wash Buffer RPE to the top of your RNeasy column. Centrifuge for 30 s in a microcentrifuge. Dispose of the flow-through fraction and place the column back onto the 2 mL microcentrifuge tube.
- 8.** Add 500 μ L of Wash Buffer RPE to the top of your RNeasy column. Centrifuge for 2 minutes at full speed in a microcentrifuge. The membrane of the RNeasy column should be dry after this step. Let it air dry for a minute or two to make sure. Transfer the RNeasy column (with RNA bound to it) to a new 1.5 mL tube.
- 9.** Add 30 μ L of RNase-free water directly onto the membrane of the RNeasy column. Wait for 5 minutes to allow the RNA to dissolve in the water. Centrifuge for 1 min. Add another 30 μ L of RNase-free water directly onto the membrane of the RNeasy column. Centrifuge again for 1 min.
- 10.** Remove a 5 μ L aliquot of your RNA sample and add it to a 500 μ L tube. Immediately freeze the rest of your sample (make sure the tube is well labeled).

Measure the absorbance of each RNA sample at 260nm and at 280nm. For pure RNA, the ratio of A_{260}/A_{280} should be between 1.7 and 2.0. Calculate the concentration of each of your RNA solutions from the A_{260} as follows:

$$A_{260} \times 0.04 = \text{RNA concentration (in } \mu\text{g}/\mu\text{L)}$$

APPENDIX A

MEASURING THE ABSORBANCE OF RNA SAMPLES USING THE NANODROP SPECTROPHOTOMETER

1. Open up the Nanodrop software on the computer that is connected to the spectrophotometer.
2. Click the **Nucleic Acids** button on the computer screen.
3. Load 2 μL of deionized water onto the sample pedestal and lower the sample arm. Click **OK** on the computer screen.
4. Wait until the spectrophotometer has completed its “initializing” procedure. For your **Sample Type**, select **RNA-40**.
5. Click on the **Blank** button to set the zero absorbance baseline for the spectrophotometer.
6. Type in the name of your first sample in the **Sample ID** box. Lift up the sample arm, and gently blot away the water sample with a piece of soft paper. Load a 2 μL aliquot of your first sample onto the sample platform and lower the sample arm. Click **Measure**.
7. Type in the name of your second sample in the **Sample ID** box. Repeat what you did in step 6 to measure the absorbance of this sample. Continue until you have measured the absorbance of all your samples.
8. Press **Print Report** to get a printout of your results.