Arabidopsis pollen RNA extraction

This RNA extraction is a modification of the Qiagen RNeasy Mini Kit protocol for isolation of total RNA from plant cells and tissues (06/2001 version). All buffers mentioned are found in the kit.

1. Collect pollen by vacuuming the flowers. We use about 20 mg of pollen per isolation.

2. Grind pollen in liquid nitrogen using a small mortar and pestle (Coors 60313). This is not easy to do, so patience is required. Grind for a couple of minutes and add more liquid nitrogen as necessary.

3. Add 450 uL of Buffer RLT to the mortar. It will freeze to the bottom of the mortar. Use the pestle to chip away the frozen buffer. Grind the buffer into a fine powder. Carefully add more liquid nitrogen and grind for a couple of more minutes, adding liquid nitrogen as necessary.

4. When you are finished grinding, add more liquid nitrogen to keep the powder frozen and carefully transfer the buffer/pollen mixture into a 1.5 mL microfuge tube. Usually, some powder remains in the mortar and it can be difficult to remove. Use a plastic transfer pipet with the bulb cut off at an angle to scoop the remaining powder into the tube. Continue adding liquid nitrogen to the mortar, allow the powder to pool in the center of the mortar and continue scooping the powder into the microfuge tube until you think you've transferred most of the sample.

5. Allow the buffer mixture to thaw and then pipet it onto a QIAshredder column (lilac). Spin at max speed (~13,000 rpm) for 2 minutes.

6. Transfer flow-through to a fresh microfuge tube. Avoid the pellet when drawing off the liquid! Add 0.5 volumes (~225 uL) of 100% ethanol. Mix by gently inverting the tube several times.

7. Transfer the solution, including any precipitate, to the RNeasy column (pink) and spin at max speed for 15 seconds. Discard the flow-through.

8. Place the RNeasy column in a fresh microfuge tube and add 700 uL of Buffer RW1. Incubate at room temperature for 5 minutes. Spin at max speed for 15 seconds. Discard flow-through.

9. Transfer column to a fresh 2 mL collection tube and pipet 500 uL of Buffer RPE onto the column. Let it sit at room temperature for 2-3 minutes. Spin at max speed for 15 seconds. Discard flow-through.

10. Repeat wash with 500 uL Buffer RPE. Spin at max speed for 2 minutes. Discard flow-through. Transfer column to a clean 1.5 mL microfuge tube and spin at max speed for 2 minutes to remove any remaining ethanol from the wash.

11. To elute RNA off of the column, pipet 50 uL of RNase-free water (provided in the kit) onto the column membrane. Let water stand for 1 minute and then spin at max speed for 1 minute. If desired, this step can be repeated (and according to Qiagen) it should be if you expect >20 ug RNA from your sample.

12. Using this method we usually collect > 50 ug of total RNA from a 20 mg of pollen.

4ug of total RNA (middle lane of gel) isolated from pollen using this method.