Panicum sp. RNA extraction using Spectrum Plant Total RNA kit from Sigma

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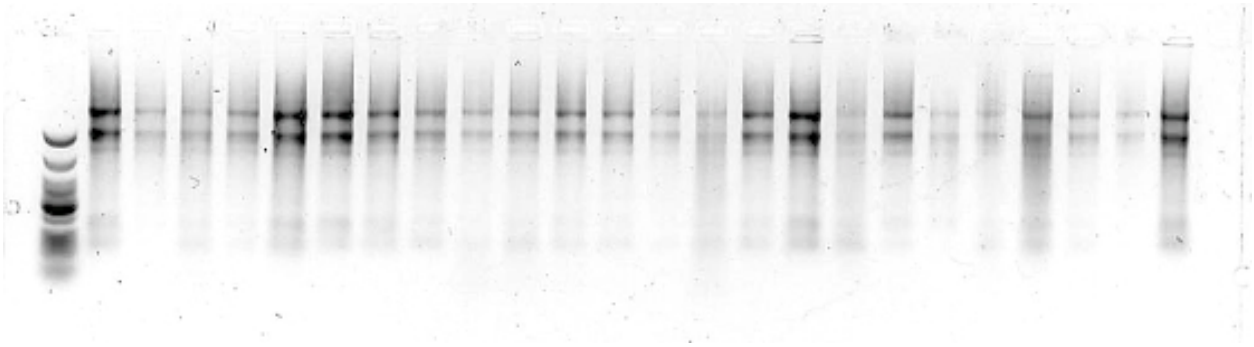
This protocol was used to extract RNA to be used in RNAsq library preps to be run in Illumina platform. This protocol has been used in another species of monocot such as Brachypodium and rice, and also in Arabidopsis. 90 samples each of three species of *Panicum* were used, obtaining following concentration and quality ratio (260/280):

Species	[ng/ul]	260/280
Panicum hallii var hallii	304 (SD 127)	2.23 (SD 0.03)
Panicum halli var *filipies*	216 (SD 92)	2.21 (SD 0.03)
Panicum virgatum	438 (SD 190)	2.14 (SD 0.04)
Total	319 (SD 168)	2.19 (0.05)



1% agarose gel showing random samples (Time run 25 min, 1 ul per sample loaded).

Kits:

1. Spectrum Plant Total RNA kit from Sigma, Catalog No. STRN250
2. On-column DNase digestion, Catalog No. DNASE70.

Before start:

1. Wash solution 2. Be sure the wash solution 2 is prepared.
2. Grind tissue: put 3-4 steel balls (clean these balls with RNase free trap and soap) put each sample, and cut the big tissue using a pin, just press until bottom. After, put two samples each time in the external hole to the grinder, using a 30

frequency and 15 seconds. Keep all time the samples in liquid nitrogen. Avoid that NL goes inside the tube because it will exploit and lost the sample. This protocol is for 100 mg of tissue powder.

3. Mark and assemble column and collection tubes. Two set to 2 ml tubes (included in the kit) and in one set hold in a filtration column (blue ring) and in the other a binding column (red ring). Mark another set of 1.5 ml tube to collect the RNA.
4. Turn on the heater at 56ºC.
5. In a styrofoam container with ice put the DNase buffer to melt.
6. Prepare the lysis solution: for 24 samples, in a 15 ml tube, mix 12.5 ml of lysis buffer (500 ul for each sample) + 125 ul of 2-ME mixture (10 ul of 2-ME for every 1 ml of lysis solution). Prepare this lysis solution in the gases extraction chamber.

Extraction protocol:

1. Put the samples in ice using a grid, in rows of 8 samples and add 500 ul of lysis solution using the fancy multichannel.
2. Vortex the samples. Put each tube in the vortex in diagonal position (nor vertical) until all tissue are in contact with the buffer (at least for 30 seconds).
3. Incubate the sample at 56ºC for 4 min.
4. Centrifuge at maximum speed for 3 min.
5. Filter lysate: Pipette the lysate supernatant (around 400 ul), into a filtration column (blue ring).
6. Centrifuge at maximum speed for 1 min.
7. Bind RNA to column: Follow protocol A. Pipette 500 ul of Binding solution into the clarified lysate and mix immediately and throughly by pipetting at least 5 times.
8. Pipette 750 ul of the mixture into a binding column (red ring).
9. Centrifuge at maximum speed for 1 min and through out the liquid. If the binding column is clogged, re-centrifuge the column for 2-3 min. If clogged persists, remove the liquid with a pipette.
10. On-column DNase digestion: pipette 300 ul of wash solution 1 into the binding column.
11. Centrifuge at maximum speed for 1 min. Decant the flow-through.
12. For 24 samples combine 250 ul of DNase (10 ul for each sample) with 1750 ul (875 ul 2x) of DNase digestion buffer (70 ul for each sample).
13. Pipette 80 ul of the mixture directly onto the center of the filter inside the Binding column and wait for 20 min at room temperature.
14. Pipette 500 ul of wash solution 1.
15. Centrifuge at maximum speed for 1 min. Through out the liquid.

16. Pipette 500 ul of the diluted wash solution 2.
17. Centrifuge at maximum speed for 30 sg. Through out the liquid.
18. Pipette 500 ul of the diluted wash solution 2.
19. Centrifuge at maximum speed for 30 sg. Through out the liquid.
20. Centrifuge at maximum speed for 1 min.
21. Transfer the column to a 1.5 ml tube.
22. Pipette 35 ul of Elution buffer and wait for 1 min.
23. Centrifuge at maximum speed for 1 min.
24. Pipette the same 35 ul again in to the column.
25. Centrifuge at maximum speed for 1 min.
26. Keep the RNA in ice to be quantified (NanoDrop or Quibit) and run in 0.8% agarose gel.