Modified Spectrum Plant Total RNA Kit Protocol

Introduction

This protocol is modified slightly from the given protocol, firstly in that we add PVP40 to help deal with polyphenolic compound formation (this is added in bulk to the whole bottle of lysis solution). Secondly, some of the prep steps are done in a slightly different order, and details are added for certain steps. Lastly, we include quantification and data entry in the same workflow.

Materials

Grinding

- 2 Styrofoam containers (coolers)
- Liquid Nitrogen
- Ladel
- Tongs
- Tweezers
- Dry Ice
- Mortar and Pestle (baked >24hrs @ >250°C)
- Nitrile Gloves
- Cloth Gloves
- RNAse Away
- Analytical Balance (Ligon Lab)
- Weigh Boat
- Beaker (baked as above)
- Kim Wipes
- Scoopula (baked as above)
- Ultra fine tip sharpie
- Eppendorf Tubes (Sterile)

Extraction and Binding

- Beta-Mercaptoethanol (2-ME)
- Fume Hood
- Weigh boat
- Scoopula (baked as above)
- Eppendorf Tubes (Sterile)
- Collection Tubes (kit)
- Heat Block
- Ultra Fine Tip Sharpie
- Columns (kit)

- Lysis Solution (kit)
- Wash Solution 1 (kit)
- Wash Solution 2 (kit)
- Binding Solution (kit)
- Elution Solution (kit)
- Tube Rack
- P100 Pipette and Filter Tips
- P100 Pipette and Filter Tips
- Vortex
- Tube Opener
- Kim Wipes
- Nitrile Glove
- Centrifuge

Quantification

- Qubit Buffer
- Qubit Reagent (caution, photosensitive)
- Qubit Tubes
- Qubit RNA Standards 1 and 2
- Eppendorf Tube (Sterile)
- Qubit
- P100 Pipette and Filter Tips
- P10 Pipette and Filter Tips

Data Entry

- Computer

Procedure

More than one day before the extraction

1. Check to make sure there are baked items to use for your extraction.

   If there are no baked items, collect the required items, and wrap them in foil. For the mortars, place foil over the top opening. For pestels wrap the bottom. For small tools make a foil pouch that holds multiple tools. Place these items in the oven in the prep room behind Temple 244 at ~250°C for at least a day.

2. Check to make sure we have dry ice (stored in the -80°C freezer in 231). If we are out tell Dr. Kovacs

3. Check to make sure we have nitrogen (stored in dewers in 231A). If we are out tell Dr. Kovacs.

4. Check to make sure we have sterile tubes.

   If we do not, fill a 750mL halfway with Eppendorf tubes then put foil over the top. Label this beaker with lab tape that says "Tubes Kovacs Date" and place it in the autoclave room (Two doors down from our lab). If you are trained, autoclave the tubes.

Less than an hour before the extraction

5. Move all of the required materials to Temple 228, and organize your work space. This is a shared working space, so try to keep it compact.

6. Review the entire protocol before extracting. Do this with your extraction partner.

7. Plug the heatblock into the outlet at the fumehood, and set it to "low"

8. Plug in the centrifuge on a lab bench, and set the temperature to 4°C, the speed to the maxium, and the time to 3 min. Then, push fast temp to cool it down.

9. Plug in and level the analytical balance.

10. Pull out and label all the tubes you will need. For 1 extraction, you will need 1 Eppendorf for your sample, and 3 collection tubes (1 empty, 1 with a red column, 1 with a blue column).

    For every two extractions (which is why we work in buddies) you will need 1 Eppendorf for the lysis solution and 1 more Eppendorf for the Qubit working solution. Make sure to handle sterile tubes with tweezers, and keep them closed when not in use. The column tubes can be finicky to close, just hold it for a bit.

Table1

	A	B	C	D
1	Se_0_1	Se_24_1	Se_48_1	Se_72_1
2	Se_0_2	Se_24_2	Se_48_2	Se_72_2
3	Se_0_3	Se_24_3	Se_48_3	Se_72_3
4	In_0_1	In_24_1	In_48_1	In_72_1
5	In_0_2	In_24_2	In_48_2	In_72_2
6	In_0_3	In_24_3	In_48_3	In_72_3
7	Fe_0_1	Fe_24_1	Fe_48_1	Fe_72_1
8	Fe_0_2	Fe_24_2	Fe_48_2	Fe_72_2
9	Fe_0_3	Fe_24_3	Fe_48_3	Fe_72_3
10	Ma_0_1	Ma_24_1	Ma_48_1	Ma_72_1
11	Ma_0_2	Ma_24_2	Ma_48_2	Ma_72_2
12	Ma_0_3	Ma_24_3	Ma_48_3	Ma_72_3

11. Prepare the Lysis Solution

    Under the fume hood, add 1.2mL of Lysis Solution from the kit, 12µL of 2-ME to an Eppendorf tube. Label this tube lysis, and put the bottle of Lysis Solution back into the kit to avoid confusion. Vortex the solution thoroughly.

12. Verify the heat block is at 56°C, adjust as needed.

13. Check the data entry see to see what samples still need processed. Keep your sample in LN2 or dry ice as long as it is out of the -80°C.

14. Fill a beaker with dry ice, and a cooler with dry ice.

15. Wipe down the workspace with RNAse away.

16. Remember, you can cut protocol time by tidying and puting things away during spins and incubations.

Extraction

17. Pour 1-2 inches of LN2 into a Styrofoam box, then place the mortar, pestle, and weigh boats into the nitrogen. Once the nitrogen stop boiling, remove the mortar and pestle.

18. Quickly break off about half a thimblefull of leaf tissue into the mortar. Be sure to replace the sample in dry ice or LN2.

Freezer rack layout

Table2

	A	B	C	D
1	Sensitive 0 hr	Insensitive 24 hr	Parents 48 hr	Sensitive 168 hr
2	Insensitive 0 hr	Parents 24 hr	Sensitive 0 hr	Insensitive168 hr
3	Parents 0 hr	Sensitive 48 hr	Insensitive 0 hr	Parents 168 hr
4	Sensitive 24 hr	Insensitive 48 hr	Parents 0 hr	RNA samples

19. CRITICAL Ladel LN2 into the mortar and begin grinding the tissue while wearing the cold temperature gloves. When the nitrogen evaporates add more. Grind to a fine powder.

20. Once the sample is gound, replace the mortar, pestle and sample back into the LN2.

21. Tare the analytical balance using a room temperature weigh boat.

22. Cool a labelled Eppendorf in LN2, then transfer it to a beaker full of dry ice.

23. Begin moving your sample to the cold weigh boat. Keep it as cold as possible.

24. Weigh 100mg of sample, and transfer it to the Eppendorf tube.

25. Transfer the Tube with the sample to the fume hood, and pipette in 500µL of prepared Lysis solution. Vortex immediately and thoroughly.

26. Incubate for 4 min at 56°C.

00:04:00

27. Make sure to close the lid and dispose of the lysis solution.

28. Spin lysate for 3 min at max speed. Make sure to point the hinge of the tube towards the top of the centrifuge.

00:03:00

29. Carefully transfer the supernatent (do not take any pellet) to the collection tube with the blue column. Close the lid and dispose of the pellet.

30. Spin lysate for 1 min at max speed.

00:01:00

31. Discard column.

32. Add 250µL of Binding solution to the flowthrough.

33. Mix by pipetting 5 times.

34. Transfer mixture to a collection tube with the red column.

35. Spin for 1 min at max speed.

00:01:00

36. Decent the flow through onto a kim wipe, and replace the column in the tube. Discard the wipe.

37. Add 500µL of Wash 1.

38. Spin for 1 min at max speed.

00:01:00

39. Decant flow through as above, replace column.

40. Add 500µL of Wash 2.

41. Spin for 1 min at max speed.

00:01:00

42. Decent flow through as above, replace column.

43. Add 500µL of Wash 2.

44. Spin for 1 min at max speed.

00:01:00

45. Decent flowthrough, replace column.

46. Spin for 1 min at max speed.

00:01:00

47. CRITICAL Transfer column to new (labelled) tube without touching the column to the sides of the tube or flowthrough.

48. Add 50µL of Elution buffer directly to the filter in the column.

49. Incubate for 1 min at room temperature.

50. Spin for 1 min at max speed.

00:01:00

51. Remove and discard column.

52. PAUSE Transfer flowthrough to dry ice. This is the RNA product.

Quantification

53. Prepare Qubit working solution. For two standards and two samples add 900µL of buffer and 5µL of reagent to an eppendorf. Vortex breifly.

54. Transfer 10µL of standard 1 and 190µL of working solution to a clean Qubit tube. Vortex and read the standard.

55. Transfer 10µL of standard 2 and 190µL of working solution to a clean Qubit tube. Vortex and read the standard.

56. Transfer 199µL of working solution and 1µLof your sample (thawed and vortexed) to a clean tube. Read the sample and record the result in the data entry file.

57. Repeat step 56 for each sample.

Data entry

58. Enter your concentration to the data entry form, along with your name.

59. Lastly, clean up 228, and return all materials to the appropriate place. Wipe down all counters with EtOH and Kim wipes.