RNA EXTRACTION – TRIZOL METHOD

Wear gloves during all the process.

DAY1:

1. Put liquid nitrogen in a mortar with the pestle inside to cool down both.

2. Ground 100 mg of plant tissue in a frozen mortar with a frozen pestle. Poor little liquid nitrogen in the mortar to ground the tissues a second time.

3. When a thin powder is available, add under the hood 1 ml of trizol in the mortar.

4. Mix trizol and powder with the pestle to constantly create a homogeneous mix.

5. When trizol become liquid, transfer all the volume in a 1.5 eppendorf tube.
Optional: 65°C water bath for 30 min, vortex per 5 min.

6. Centrifugation 1: 3500 rpm, 4°C, 10 min

7. Transfer the supernatant in a second tube. Add 200 ul of chloroform. Mix. Wait 10 min at room temperature

8. Centrifugation 2: 9000 rpm, 4°C, 15 min

9. Remove very carefully the upper phase (clear) without to remove the interphase of the lower phase (red). Mainly 500 ul of the volume should be removed. Put the upper phase in another eppendorf tube. Add 500 ul of isopropanol. Mix.

10. Store at -20^{o}C, overnight.

DAY2:

11. Centrifugation 3: 14000 rpm, 4°C, 40 min

12. After this centrifugation, you should have a pellet in the bottom of the tube. Remove the supernatant. Add 400 ul of EtOH 70% in the tube to wash the pellet.

13. Centrifugation 4: 12000 rpm, 4°C, 5 min

14. Remove the supernatant.

15. Let the pellet dry.

16. Resuspend it in 100 ul H2O.

17. Add 10 ul of AcNA.

18. Incubate 10 min on ice

19. Add under the hood 100 ul of chloroform.

20. Mix strongly and centrifuge 10 min at 9000 rpm (4°C if possible; or RT).

21. Put the upper phase in a new eppendorf tube RNase-free (be careful to isolate the maximum without to pipette the interphase and the lower phase).

22. Add 200 ul of EtOH 100%.

23. Put the tube at -20°C overnight.

DAY3:

24. Centrifuge the tube at 4°C, 40 min, 14000 rpm. You should have a pellet.

25. Remove the supernatant. Add 300 ul EtOH 70%.

26. Centrifuge the tube at 4°C, 10 min, 12000 rpm.

27. Remove the supernatant. Let the pellet dry completely.

28. Resuspend the pellet with 30 ul of water RNase-free.

If needed

Turbo DNase treatment of total RNA (kit:TURBO DNA-free™ Kit):

30 ul total RNA (concentration >200 ng/ul)
3 ul of Turbo DNase (Ambion, ref# 1907)
4 ul of Turbo DNase buffer (10x)
H2O qsq 40 ul
1H, 37 °C
Add 8 ul of Turbo DNase Stop buffer
MIX VERY WELL
5 min on the bench, flick the tube 2-3 times during the incubation period
Centrifugation: 1 min 30 sec, 10000 rpm
Put the supernatant VERY carefully in another tube.
Centrifuge the tube agai, put the supernatant in the third tube to get the free-gDNA RNAs are in the supernatant.

Qubit procedure

1. Use the Qubit® RNA HS/BR Assay Kit and Qubit™ assay tubes (500 tubes, Invitrogen Cat. no. Q32856)
2. Dilute the Turbo DNase-treated RNA by 1:10 (using 0.5ul RNA plus 4.5ul nuclease-free water), as the tested sample
3. The following steps according to the protocol of the kit including calibration of the Qubit system.
4. The calculation is the same with the kit, however, since the RNA samples was diluted 10 times before measurement, the concentration indicated by the Qubit system must be multiplied by 10.
5. According to the concentration calculated above, aliquot and dilute the sample into the needed volume and concentration.
6. Run a gel to validate the quality of the sample
7. Analyze the level of purity of the sample using Nanodrop.