RNA extraction from plant samples

Principle

RNA extraction from plant samples for subsequent synthesis of cDNA and Real time PCR analysis. This is done using the QIAGEN RNeasy plant mini kit. This protocol is based on the protocol described in the RNeasy Mini Handbook (available at the Qiagen website http://www.qiagen.com/knowledge-and-support/resource-center/resource-download.aspx?id=14e7cf6e-521a-4cf7-8cbc-bf9f6fa33e24&lang=en)

Equipment and Reagents

Machine/Product	Reference (Company, Type, …)
Sterile, RNAse-free filtertips	1 ml, 200 µl, 10 µl filter tips
Centrifuge – cooled	Eppendorf 5415R with F45-24-11 rotor
Mixer Mill / Cryo Mill	Retsch MM 400 with 2x PTFE Adapter rack for 10 reaction vials 1.5 and 2.0 ml.
Microtube Vortex	IKA Vortex 1
Eppendorf tubes	Safe-lock®, 1.5 and 2.0 ml; RNAse/DNAse free.
Liquid Nitrogen (+ thermos jar)	
3 mM diameter stainless steel balls	R22.455.0002 Verder NV
Mixer Mill / Cryo Mill	Retsch mM 400 with 2x PTFE Adapter rack for 10 reaction vials 1.5 and 2.0 ml.
RNeasy plant mini kit (50)	 QIAGEN Cat No. 74904
ß-mercaptoethanol 14.3 M	 Sigma M3148
Ethanol 96-99 %	

Protocol

Handling and storage of starting material

- Samples are immediately flash frozen in liquid nitrogen and stored at -80 °C as soon as they are harvested. Frozen tissues should not be allowed to thaw during handling or weighing. The relevant procedures should be carried out as quickly as possible. Frozen samples are stable for months.

Disruption using a mortar and pestle

- Complete disruption of cell walls is absolutely required to release all the RNA contained in the sample. Incomplete disruption results in significantly reduced yields.
- Burn out the mortar and pestle with ethanol. Leave to cool a few minutes. Add liquid nitrogen to the mortar and pestle to cool the down.
- Transfer the sample in the liquid nitrogen in the mortal. Quickly grind the sample when the liquid nitrogen is almost all evaporated.
- Transfer the sample powder in an Eppendorf tube.
- Immediately add the 450 µl RLT buffer from the first step of the isolation protocol.

Disruption using the shredder

- Cleaning the shredder beads: Put enough 3 mM diameter stainless steel beads in a porcelain mortar and add a small amount of ethanol. Carefully ignite the ethanol to flame-sterilize the beads. Leave to cool.
- Transfer the plant samples (±100 mg) from the low temperature freezer (-80 °C) to the liquid nitrogen. Use expanded polystyrene floats to keep them organized.
- Pre-cool Eppendorf adaptors of the mixer mill in liquid nitrogen. The adaptors are cool when they don't "bubble" anymore.

- Place in Mixer mill adapters, and shred for 3,5 minutes at 30Herz. You can shred 10 samples at once (5 per block, see figure). If more tubes are placed, the risk exists that the caps of the outer tubes will snap.

RNA isolation Protocol

- After shredding, immediately add 450 µl **RLT buffer** and vortex well with the pipette, then transfer all to the lilac shredder filter. Centrifuge for 2 minutes at full speed (13000 rpm)
- Discard the lilac shredder filter and carefully transfer the supernatant filtrate (do not disturb the pellet) to an Eppendorf containing 250 µl ethanol 99 %. Vortex with the pipette and transfer to the resin filter (pink). Centrifuge for 15 seconds at 10000 rpm.
- Discard the filtrate (not the tube) and add 700 µl **RW1 buffer** to the filter. Centrifuge for 15 seconds at 10000 rpm.
- Discard the filtrate (and the tube) and transfer the resin filter to a new 2 ml tube. Add 500 µl **RPE buffer** to the filter and centrifuge for 15 seconds at 10000 rpm.
- Discard the filtrate (not the tube) and again, add 500 µl **RPE buffer** to the filter. Centrifuge for 1 minute at full speed (13000 rpm).
- Discard the filtrate (and the tube) and transfer the resin filter to a new Eppendorf tube. Centrifuge for 1 minute at full speed (13000 rpm).
- Discard the filtrate (and the tube) and transfer the resin filter to a new Eppendorf tube. Add 30 µl **RNase-free water** and centrifuge for 1 minute at 10000 rpm.
- Do not discard anything and again add 30 µl **RNase-free water** and centrifuge for 1 minute at 10000 rpm.
- Vortex the solution with the pipette and transfer 5 µl in a new RNase-free microtube for nanodrop (1.5 µl) and bioanalyzer (1 µl) analysis.

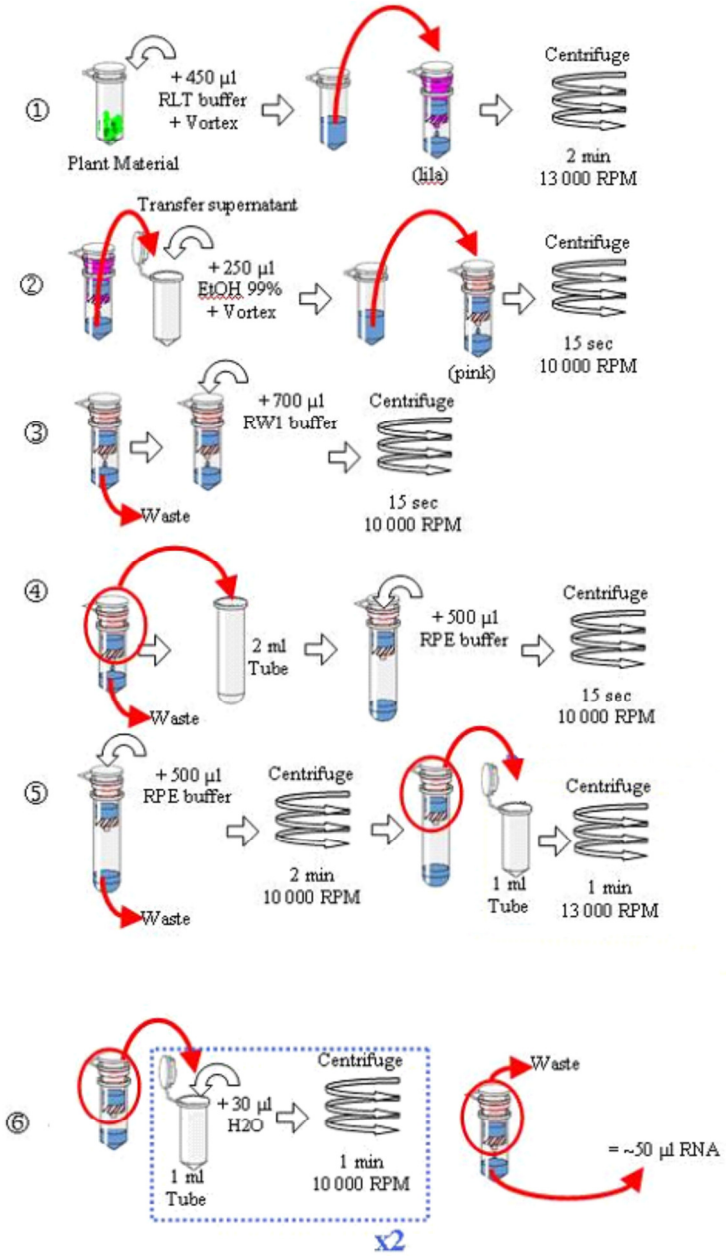

Figure 1: Schematic overview of the Protocol for Isolation of Total RNA from plant cells and filamentous fungi using Rneasy plant mini kit.