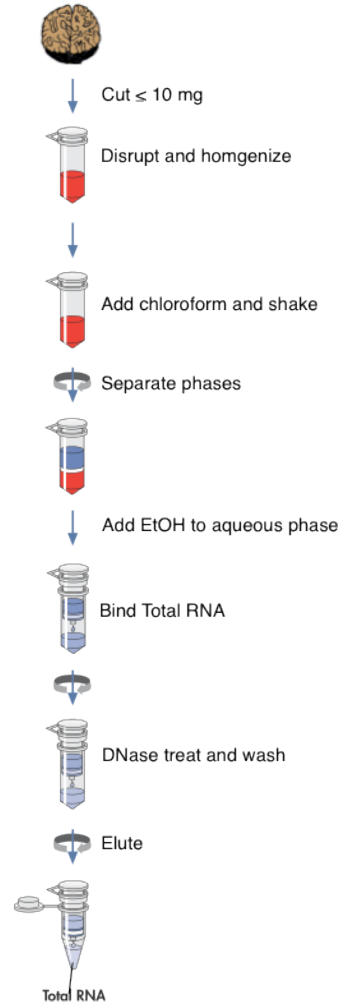
Extract Total RNA from Lipid Tissues

This process disrupts lipid tissues with a pellet pestle in the presence of a mono-phasic solution containing chaotropic denaturants phenol and guanidine thiocyanate. The solution is homogenized using a syringe and 21 g needle, and separated into an aqueous phase and organic phase with the addition of chloroform. The RNA is recovered from the aqueous phase and purified using silica-based membrane technology with on column DNase digestion. RNA molecules < 200 nt are selectively excluded.



Equipment

- Thermoregulated microcentrifuge (with rotor for 2 ml tubes)
- Vortex mixer
- Pellet pestle motor
- Sterilized forceps
- Digital laboratory scale

Consumables

- RNase-free water
- Disposable gloves
- Ethanol
- Sterile, RNase-free pipet tips
- DNase kit
- MinElute columns
- RNase-free tubes
- Disposable sterile pellet pestle probes
- Sterile petri dish
- Stainless steel surgical blades, sterile
- Molecular-biology grade chloroform

Preparation

- 70% ethanol
- DNase I stock solution
- Chill microcentrifuge to 4ºC.
- Buffer RPE

Procedure

___ 1 Remove tissue from -80ºC[1]. Let thaw on ice.
___ 2 Transfer tissue from storage container onto petri dish using forceps. Excise ≤ 10 mg (determine amount by weighing) and transfer to 1.5 ml RNase-free tube.
___ 3 Add 750 ul Qiazol lysis reagent.
___ 4 Manually subject tissue to disruption with clean pellet pestle probe.
___ 5 Attach probe to motor and grind for 30 s or until lysate uniformly homogeneous.
___ 6 Add an additional 250 ul Qiazol lysis reagent.
___ 7 To ensure complete homogenization and to shear high-molecular weight cellular component, pass sample through clean 21 g syringe needle 10 times.
___ 8 Let homogenate sit at RT (15-25 ºC) for 5 min for complete dissociation of nucleoprotein complexes.
___ 9 Add 200 ul chloroform. Vortex vigorously for 15 s.
___ 10 Let homogenate sit at RT (15-25 ºC) for 3 min.

[1] Tissue should be preserved in RN*Alater* RNA Stabilization solution.

___ 11 Centrifuge at 12,000 x g for 15 min at 4°C. After centrifugation, heat the centrifuge to room temperature (15–25°C).
___ 12 Transfer upper aqueous phase (usually 600 ul) to clean 1.5 ml RNase-free tube.
___ 13 Add 1 volume (usually 600 ul) 70% EtOH, vortex, and briefly spin[2].
___ 14 Transfer sample (700 ul) to an RNeasy MinElute spin column placed in a 2 ml collection tube. Let sit for 2 min at RT (15-25 ºC). Close lid, and centrifuge at 10,000 rpm for 30 s at RT (15-25 ºC). Discard flow-through.
___ 15 Repeat step 14 using the remainder of the sample. Discard flow-through.
___ 16 Add 350 ul Buffer RW1 to spin column. Close lid, and centrifuge at 10,000 rpm for 30 s at RT (15-25 ºC). Discard flow-through.
___ 17 Add 70 ul Buffer RDD to 10 ul DNase I stock solution. Mix by gentle inversion, and briefly spin.
___ 18 Transfer 80 ul of DNase mix directly to the center of the spin column membrane. Incubate at RT (15-25 ºC) for 15 min.
___ 19 Add 350 ul Buffer RW1 to spin column. Close lid, and centrifuge at 10,000 rpm for 30 s at RT (15-25 ºC). Discard flow-through.
___ 20 Add 500 ul Buffer RPE to spin column. Close lid, and centrifuge at 10,000 rpm for 30 s at RT (15-25 ºC). Discard flow-through.
___ 21 Add an additional 500 ul Buffer RPE to spin column. Close lid, and centrifuge at 10,000 rpm for 2 min at RT (15-25 ºC). Discard flow-through and collection tube.
___ 22 Place spin column in a new 2 ml collection tube. Open the lid of the spin column (orient so that they point in the direction opposite the rotation of the rotor; leave at least one empty position between columns), and centrifuge at 13,200 rpm for 5 min at RT (15-25 ºC).
___ 23 Place spin column in a new 1.5 ml collection tube. Let sit for 5 min at RT (15-25 ºC) with lid open to completely evaporate residual ethanol.
___ 24 Add 54 ul RNase-free water directly to the center of the spin column membrane. Let sit at RT (15-25 ºC) for 2 min, and centrifuge at 13,200 rpm for 1 min at RT (15-25 ºC).
___ 25 Pipet flow-through from the collection tube (52 ul) back onto the spin column membrane. Let sit at RT (15-25 ºC) for 2 min, and centrifuge at 13,200 rpm for 1 min at RT (15-25 ºC).

[2] Avoid prolonged centrifugation, which will drive the RNA through the ethanol solution to the wall of the tube.