Isolation of RNA

1.) Weigh sample to between 50 and 100 mg. Don't let sample defrost!
2.) Put sample in motor and pestal
3.) Add liquid nitrogen
4.) Grind sample
5.) Pour sample and liquid nirogen into eppendorf tube
6.) Wait for liquid nitrogen to evaporate
7.) Add **450 uL of RTL buffer** and **4.5 uL B-ME**
    (if making buffer solution ahead of time: 1 ml buffer x 10 uL B-Me)
8.) Vortex
9.) Incubate at 56 Celsius for 1-3 minutes
10.) Pipet all of the lysate (approximentaly 550 ul) into the **lilac QIA shredder** and put that into a 2 ml eppendorf tube. (For this step cut off end of pipet tip with ethanol-steralized scissors)
11.) **Centrifuge for 2 min** max speed. (may need to snip the pipet tip)
12.) Pipet the flow through supernatant to a new microcentrifuge tube without disturbing the pellet.
13.) Add **225 ul ethanol** to the supernatant, and mix by pipetting. Precipitate may form. No centrifuging!!! Go immediately to next step!
14.) Add **650 ul** of above to an **Rneasy mini column** (pink) placed in a 2 ml collection tube (supplied).
15.) **Centrifuge for 15 s** at > 10, 000 rpm/min.
16.) **Discard** whatever lands in the centrifuge tube, but do not throw away the tube.
17.) Add **700 ul buffer RW1** to the pink column.
18.) **Centrifuge for 15s** at 10000 rpm. Now throw away the tube and the flow through.
19.) **Transfer** the pink column to a new 2 ml collection tube.
20.) Pipet **500 ul of buffer RPE** to the pink column.
21.) **Centrifuge for 15s** at 10000 rpm.
22.) **Discard** the flow through.
23.) Add **500 ul RPE** to the pink column.
24.) **Centrifuge 2 min** at 10000 rpm.
25.) **Transfer** RNeasy column into a new 2 ml tube and **centrifuge for 1 min.**
26.) **Repeat**.
27.) **Transfer** the RNeasy column to a new 1.5 ml collection tube (supplied) and
28) Pipette **30-50 ul RNase free water** directly into the RNeasy membrane. Don't let it touch the sides.
28.) **Centrifuge for 1 min** at 10000 rpm.
29.) **Store** the RNA in a –20 - -70 celsius freezer.

Quantitation of RNA

1.) Wash cuvette with .1M NaOH, 1mM EDTA, followed by washing with Rnase-free water. (Follow the solutions guide). It has to be stored for 12 hrs.
2.) 10 ul of RNA sample + 490 ul distilled water (1/50 dilution).
3.) Measure absorbance in a 1 ml cuvette at 260 nm.

Concentration of RNA sample= 40 x Absorbance x 50.

Run a 1 % agarose gel (If you want to). 10 ul sample + 2 ul 10x loading dye.