\*Procedure is in our lab notebook as well\*

\*\*This procedure is the baseline procedure, the one Richard had us do includes some minor changes/edits\*\*

**RNeasy Plant Mini Kit Protocol**

**Preparation**

1. You will need **450 uL** of **Buffer RLT** per sample; calculate the total volume of **Buffer RLT** for all of your samples (27 samples \* 450 uL = **12.15 mL Buffer RLT**).
2. Check **Buffer RLT** to see if 𝛃-**ME** has been added within the last month.
3. If no 𝛃-**ME** has been added, or if longer than a month, find new **Buffer RLT**  and add **10 uL** 𝛃-**ME** per **1 mL Buffer RLT** (𝛃-**ME** is a neurotoxin, so add in the vacuum chamber).
4. You will need **1 mL** of **Buffer RPE** per sample; calculate the total volume of **Buffer RPE** for all your samples.
5. Check **Buffer RPE** to see if **ethanol** has been added.
6. If no **ethanol** has been added, add 4:1 (**ethanol : Buffer RPE** concentration).
7. You will need **700 uL** of **Buffer RW1** per sample; calculate the total amount of **Buffer RW1** needed.

**Procedure**

1. Add **450 uL** of **Buffer RLT** to each tube containing maximum **100 mg** plant material.
2. Add 3-4 ball bearings to each tube
3. Place tubes in **Mini-G** for 4-5 minutes
4. Add **0.5 volume** of **95-100% ethanol** to the **supernatant** (plant tissue), and pipette up and down to mix.
5. Transfer the sample (**~650 uL**) to an **RNeasy Mini spin column** (special tube, pink)
6. Close the **spin column** lid and **centrifuge** for **15s** at **10,000 rpm**.
7. Discard **flow-through**.
8. Add **700 uL Buffer RW1** to the **spin column**.
9. Close the **spin column** lid and **centrifuge** for **15s** at **10,000 rpm**.
10. Discard **flow-through**.
11. Add **500 uL Buffer RPE** to the **spin column**.
12. Close the **spin column** lid and **centrifuge** for **15s** at **10,000 rpm**.
13. Discard **flow-through**.
14. Add **500 uL Buffer RPE** to the **spin column**.
15. Close the **spin column** lid and **centrifuge** for **2 min** at **10,000 rpm**.
16. Dry the **membrane** centrifuging **spin column** in new **collection tube** for 1 min.
17. Place the **RNeasy spin column in a** sterile **1.5 mL Eppendorf tube**.
18. Add **50 uL** **RNase-free-water** directly to the **membrane**.
19. Close the **spin column** lid and **centrifuge** for **1 min** at **10,000 rpm** to elute **RNA**.
20. Pipette the **50 uL eluate** directly onto the **membrane**, using the same **2 mL collection tube**.
21. Close the **spin column** lid and **centrifuge** for **1 min** at **10,000 rpm**.
22. Freeze the **50 uL RNA** solution at **-80 C** to store.

**Calculating the Quality of RNA**

1. Use the NanoDrop
2. Make sure sample are in order on ice (so RNA degrade is slow)
3. Use autoclaved water to clean machine with a tissue.
4. Start the NanoDrop software and blank the RNase free water. Be sure to set it to read RNA, not DNA.
5. Pipette 1-2 uL of RNA onto NanoDrop.
6. Close the NanoDrop lid and click measure on the computer screen.
7. Record data.
8. Good sample will have a 260/280 ratio of 1.8-2 (above 2 is perfect for RNA).
9. If samples are good, you can move onto qPCR (Real-Time PCR)

**Glossary**

* **Buffer RLT** - Lysis buffer, used to break cells open.
* 𝛃-**ME** - beta-mercaptoethanol, reducing agent that will irreversibly denature RNases by reducing disulfide bonds and destroying the native conformation required for enzyme functionality.
* **Buffer RPE** - Washing buffer, main purpose is the remove traces of salt.
* **Buffer RW1** - Washing buffer that removes the other contaminants.
* **RNase-free-water** - Fancy name for autoclaved water.