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Qiagen AllPrep 96 DNA/RNA protocol for bee abdomens (FFAR) V.1

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Ponisiolab

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Jocelynz 2021. Qiagen AllPrep 96 DNA/RNA protocol for bee abdomens (FFAR). **protocols.io** https://protocols.io/view/qiagen-allprep-96-dna-rna-protocol-for-bee-abdomen-bxxippke

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Before beginning protocol

1 DTT (2M) **Fisher Scientific Catalog #50-198-452** must be added to Buffer RLT **Qiagen Catalog #79216** before use. Add **20 µL** **20 Molarity (M)** DTT (2M) **Fisher Scientific Catalog #50-198-452** per **1 mL** Buffer RLT **Qiagen Catalog #79216** . Stock solution of

DTT (2M) **Fisher Scientific Catalog #50-198-452** should be prepared fresh or frozen in single use aliquots. Buffer RLT **Qiagen Catalog #79216** containing DTT (2M) **Fisher Scientific Catalog #50-198-452** can be stored at room temperature for up to 1 month.

DTT (2M) **Fisher Scientific Catalog #50-198-452** is highly toxic, review SDS thoroughly and dispose of as hazardous waste

Buffer RLT **Qiagen Catalog #79216** Buffer RW1 **Qiagen Catalog #1053394** Buffer AW1 **Qiagen Catalog #19081** contain guanidine salt - do not mix with bleach

Buffer RPE **Qiagen Catalog #1018013** concentrate can be disposed in the sink in small volumes – no more than 100 g solute per day, flush with 10-20 fold water thoroughly after sink disposal.

Buffer Manage and dispose of AW2 **Qiagen Catalog #19072** as hazardous waste

Prepare samples 18m

2 UV sterilize supplies for 2 96 well plates: stainless steel beads, 8 reagent reservoirs, 2 collection microtube plate (light blue) and strip caps (clear), 2 brand new s-blocks, 4 more s blocks, 4 elution microtube plates (dark blue) and strip caps (white), and 96 well microplates (for aliquots). Label foil lids for aliquots. Turn on water bath and set to **70 °C**

3 Add 1 stainless steel bead to each well of collection microtube plate

4 Working on dry ice to prevent samples from thawing, add 1 bee abdomen to each collection microtube. Enter each sample ID into your plate map as you work and keep used vials stored in order.

5 Buffer

Remove from dry ice and immediately add RLT **Qiagen Catalog #79216**

Check reagent bottle to ensure
DTT (2M) **Fisher Scientific Catalog #50-198-452** has been added before use

5.1 For larger bee abdomens such as melissodes, add **500 µL**
Buffer
RLT **Qiagen Catalog #79216**

5.2 For smaller bee abdomens such as lasioglossum, add **350 µL**
Buffer
RLT **Qiagen Catalog #79216**

Seal with strip caps and place in tissue lyser, placing a folded kim wipe between strip cap tops

6 and adapter to ensure a tight seal

TissueLyser II
Bead Mill
QIAGEN 85300

TissueLyser Adapter Set 2 x 96
Adapter set
Qiagen 69984

7 Lyse at **25 Hz** for **00:02:00** 2m

Depending on size of sample, you may need to adjust speed and time

8 Remove plates from lyser and adapters, rotate plates 180 degrees, and place back in tissue lyser

9 Lyse at **25 Hz** for **00:02:00** 2m

Depending on size of sample, you may need to adjust speed and time

10 Centrifuge **3486 x g, 20-25°C, 00:07:00** 7m

Eppendorf™ 5810R Centrifuge
Centrifuge
Eppendorf **02-262-8187**

11 Open a brand new s-block and place DNA filter plate on top

Do not reuse an old s-block for this step!

12 Transfer **350 µL** supernatant to DNA filter plate and seal with AirPore tape

13 Centrifuge **3478 x g, 20-25°C, 00:07:00** . Check that all liquid has flowed through columns, repeating centrifugation if needed 7m

**** DO NOT DISCARD FLOWTHROUGH****

14 Place DNA filter plate on new s-block and put aside at room temperature to **4 °C** for later use

You may reuse and old (sterilized) s-block for this step and all future steps

RNA purification 38m

15 Place RNA filter plate on s-block

16 Add **350 µL** 70% Ethanol **Contributed by users** to s-block containing flowthrough from step 13. Pipette up and down 3 times to mix.

17 Transfer samples to RNA filter plate. Seal with AirPore tape

18 Centrifuge **3486 x g, 20-25°C, 00:07:00** , discard flowthrough 7m

19 Buffer
Add **800 µL** RW1 **Qiagen Catalog #1053394** and seal with AirPore tape

20 Centrifuge **3486 x g, 20-25°C, 00:07:00** , discard flowthrough 7m

21 Buffer
Add **800 µL** RPE **Qiagen Catalog #1018013** and seal

22 Centrifuge **3486 x g, 20-25°C, 00:16:00** , discard flowthrough 16m

23 Place RNA filter plate on top of elution microtube plate

24 Add **60 µL** RNase-free water directly to center of each filter membrane and seal

Use **45 µL** - **70 µL** RNase-free water depending on desired DNA yield/concentration

25 Incubate at room temperature **00:01:00** 1m

26 Centrifuge **3486 x g, 20-25°C, 00:07:00** 7m

27 Repeat steps 23-26, eluting into same microtube plate

28 Distribute product into **10 µL** aliquots and seal with labeled foil lids. Seal remaining product in elution microtube plates with white strip caps

29 Store at **-20 °C** - **-80 °C**

DNA purification 35m

30 Buffer
Place EB **Qiagen Catalog #19086** in **70 °C** water bath

Place reagent bottle in water bath float - do not fully submerge

31 Get out your DNA filter plate and s-block from step 14

32 Buffer
Add **800 µL** AW1 **Qiagen Catalog #19081** and seal with AirPore tape

33 Centrifuge **3486 x g, 00:07:00** , discard flowthrough 7m

34 Buffer
Add **800 µL** AW2 **Qiagen Catalog #19072** and seal

35 Centrifuge **3486 x g, 00:16:00** , discard flowthrough 16m

36 Place DNA filter plate on top of new elution microtube plate

37 Buffer
Add **50-100 µL** preheated EB **Qiagen Catalog #19086** directly to the center of each filter membrane and seal

38 Incubate at room temperature for **00:05:00** 5m

39 Centrifuge **3486 x g, 00:07:00** 7m

40 Repeat steps 36-39, eluting into same plate

41 Distribute product into **10 µL** aliquots and seal with labeled foil lids. Seal remaining product in elution microtube plates with white strip caps

42 Store at **-20 °C** - **-80 °C**