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Qiagen DNeasy 96 blood & tissue protocol for bee guts

Lauren Ponisio[1], Jocelyn Zorn[1]

[1]University of Oregon

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ABSTRACT

Tissue protocol for bee guts.

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Day 1 1h 6m 22s

1 UV sterilize supplies for 2 96 well plates worth of extractions: 1 50mL centrifuge tube, 1 15mL centrifuge tube, 1 box 1,000uL pipette tips, 2 boxes 200uL pipette tips, 24 elution microtube strip caps, steel beads, zirconia beads, and 1 reagent reservoir

2 Combine **36 mL** Buffer ATL (tissue lysis buffer) **Qiagen Catalog #19076** and **4000 µL** Proteinase K (2 ml) **Qiagen Catalog #19131** in 50mL centrifuge tube, invert gently to mix

3 Add **200 µL** ATL + Proteinase K solution to each sample

4 Add ~ **100 µL** zirconia beads and 2 steel beads to each sample

5 Place plates in tissue lyser and run at **30 Hz** for **00:02:30** , then rotate plates 180 degrees and lyse at **30 Hz** for an additional **00:02:30** 5m

TissueLyser II
Bead Mill
QIAGEN 85300

TissueLyser Adapter Set 2 x 96
Adapter set
Qiagen 69984

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6 Centrifuge on "short" for **00:00:07** then shake by hand for **00:00:15** 22s

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

7 Incubate **56 °C** for **01:00:00** with weight on top of plate 1h
Note: remove from incubator carefully with weight on top so that caps do not pop off

8 Place plates in tissue lyser and run at **30 Hz** for **00:01:00** 1m

9 Incubate **56 °C** overnight with weight on top of plate

Day 2 53m 44s

10 UV sterilize supplies: 4 reagent reservoirs, 24 elution microtube strip caps (clear), 2 boxes 1,000uL tips (plus some extra), 6 boxes 200uL tips (plus some extra), 2 s-blocks, 2 elution microtube plates (dark blue) and 24 strip caps (white), and 6 96 well microplates (for aliquotting final product). Label 6 foil microplate lids for aliquots

11 Carefully remove plates from incubator, leaving the weight on top, and allow to sit at room temperature for a few minutes before carefully removing weight to ensure strip caps do not pop off

12 Shake plates by hand for **00:00:15** , rotating plates halfway through 15s

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13 Centrifuge on "short" for **00:00:07** 7s

14 Add **410 µL** ALE to each sample

15 Seal with new caps and shake by hand for **00:00:15** , rotating plates halfway through 15s

16 Centrifuge on "short" for **00:00:07** 7s

17 Place DNeasy filter plates on top of s-blocks

18 Transfer lysate to DNeasy filter plate and seal with AirPore tape

19 Centrifuge **3486 x g, 00:13:00** . Discard flowthrough 13m

20 Add **500 µL** Buffer AW1 **Qiagen Catalog #19081** to each sample, seal with AirPore tape

Buffer AW1 contains guanidine salt - do not mix with bleach

21 Centrifuge **3486 x g, 00:08:00** . Discard flowthrough 8m

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22 Buffer
Add **500 µL** AW2 **Qiagen Catalog #19072** , do not seal

Manage and dispose of buffer AW2 as hazardous waste

23 Centrifuge **3486 x g, 00:20:00** . Discard flowthrough 20m

24 Repeat steps 22 and 23

25 Place DNeasy filter plate on top of elution microtube plate (dark blue)

26 Buffer
Add **55 µL** AE **Qiagen Catalog #19077** to each sample

27 Incubate at room temperature for **00:01:00** 1m

28 Centrifuge **3486 x g, 00:05:00** 5m

29 Aliquot entirety of product into 3 96 well microplates per DNA plate, seal with labeled foil lids and store at **-20 °C**

30 Buffer
For a second backup elution, add **50 µL** AE **Qiagen Catalog #19077** to each sample

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31 Incubate at room temperature for **00:01:00** 1m

32 Centrifuge **3486 x g, 00:05:00** 5m

33 Seal with white strip caps

34 Store at **-20 °C**

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