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 We use this protocol and it's working

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Honeybee DNA extractions in 96-well plates

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MATERIALS

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3.2 mm stainless steel beads, RNase free **Contributed by users Catalog #NEXSSB32-RNA**

Graduated Safelock Microcentrifuge Tubes, 2.0mL, sterile **Thermo Fisher Catalog #3459IW**

ZymoBIOMICS Lysis Solution **Zymo Research Catalog #D4300-1-150**

Quick-DNA Magbead Plus Kit **Zymo Research Catalog #D4082**

RNase A solution in 10 mM Tris-HCl, pH 7.5, 15 mM NaCl at a concentration of 10 mg/mL:

250 mg RNase A
 250 µL 1 M Tris-HCl pH 7.5
 75 µL 5 M NaCl
 Make up to 25 mL

Proteinase K solution in 50 mM Tris, pH 8, 3 mM CaCl₂, 50% Glycerol at a concentration of 20 mg/mL:

100 mg Proteinase K
 250 µL 1 M Tris-HCl pH 8
 6 µL 2.5 M CaCl₂
 Make up to 5 mL

Prepare lysate for extraction

1 Prepare lysate for extraction

1.1 Dissect e.g. 8 drone heads for a single sample.

Pool the dissected material into a 2 mL safe-lock tube.

Add 4 3.2 mm **stainless steel** ball bearings and 500 µL PBS.

1.2 Homogenise the tissue for 90 seconds at 20 Hz using a Mixer Mill or Tissue Lyser.

00:01:30

1.3 Centrifuge the homogenate for 10 minutes at 16,000 g and remove and discard the top layer (i.e. PBS).

16000 x g, 21°C, 00:10:00

1.4 Transfer about 150 mg of the tissue paste to a 96-well plate for lysis.

The remaining paste can be stored at -80°C and used for subsequent extractions.

1.5 Prepare 300 µL lysis buffer per well.

A	B
Lysis solution	287 µL
RNase solution (10 mg/mL)	3 µL
Proteinase K solution (20 mg/mL)	10 µL
Total	300 µL

Add the 300 µL lysis buffer to each well of tissue plate.

1.6 Seal the plate and mix on the plate shaker. Make sure the paste is resuspended.

2h 30m

Incubate at 55°C for 150 minutes, shaking at 1000 rpm (or shake the plate for one minute every 30 minutes).

🕒 02:30:00

🌡️ 55 °C

1.7 Spin the plate for 10 minutes at 1,000 RPM.

🌀 1000 rpm, 15°C, 00:10:00

1.8 Transfer 200 µL of lysate to a new extraction plate.

DNA extraction

2 Set up the reagents from the ZYMO *Quick*-DNA Magbead Plus Kit

2.1 Add the following reagents to the 7-position ReservoirRack

Reagent	Reservoir volume (mL)	8	16	24	32	40	48	56	64	72	80	88
1: (490 µL MBB : 10 µl beads)	100	465 7	865 7	126 57	166 57	206 57	246 57	286 57	326 57	366 57	406 57	446 57
2: empty												
3: DNA pre-wash buffer	100	785 7	150 57	222 57	294 57	366 57	438 57	510 57	582 57	654 57	726 57	798 57
4: g-DNA wash buffer	100	785 7	150 57	222 57	294 57	366 57	438 57	510 57	582 57	654 57	726 57	798 57
5: g-DNA wash buffer	100	785 7	150 57	222 57	294 57	366 57	438 57	510 57	582 57	654 57	726 57	798 57
6: Water (for rinse)	100	705 7	134 57	198 57	262 57	326 57	390 57	454 57	518 57	582 57	646 57	710 57
7: Tris-HCl	10	118 3	158 3	198 3	238 3	278 3	318 3	358 3	398 3	438 3	478 3	518 3

3 Run the epMotion protocol **88x Quick-DNA Magbead Plus premixed beads DNA rinse**.

Because the rinse step is programmed once for each plate column, the protocol has to be copied and modified for each run.

3.1 The master program is at Dearden/Tom/Quick-DNA Magbead Plus/88x Quick-DNA Magbead Plus premixed beads DNA rinse.

Copy that file to your Runs directory and change the name. Use a name that includes today's date.

3.2 Change the sample number at steps 1 and 55.

Delete rinse steps between 42 and 52, leaving only the steps for columns that have samples in them.

3.3 Run the protocol with level sensing enabled.