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Honeybee DNA extractions in 96-well plates

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1 Works for me dx.doi.org/10.17504/protocols.io.bbayiifw

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MATERIALS TEXT

MATERIALS

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.

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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

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Reagent	Reservoir volume (mL)	8	16	24	32	40	48	56	64	72	80	88
1: (490 µL MBB : 10 µl beads)	**100**	**4657**	**8657**	**12657**	**16657**	**20657**	**24657**	**28657**	**32657**	**36657**	**40657**	**44657**
2: empty												
3: DNA pre-wash buffer	100	7857	15057	22257	29457	36657	43857	51057	58257	65457	72657	79857
4: g-DNA wash buffer	100	7857	15057	22257	29457	36657	43857	51057	58257	65457	72657	79857
5: g-DNA wash buffer	100	7857	15057	22257	29457	36657	43857	51057	58257	65457	72657	79857
6: Water (for rinse)	100	7057	13457	19857	26257	32657	39057	45457	51857	58257	64657	71057
7: Tris-HCl	10	1183	1583	1983	2383	2783	3183	3583	3983	4383	4783	5183

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.

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