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Low-input DNA extractions in 96-well plates

 Forked from [Honeybee DNA extractions in 96-well plates](#)

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Protocol status: Working

We use this protocol and it's working

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Materials

MATERIALS

✕ 3.2 mm stainless steel beads, RNase free **Catalog #NEXSSB32-RNA**

✕ 96 Well 0.8mL Plate (Bulk) **Thermo Fisher Catalog #AB0859**

✕ 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 μ L 1 M Tris-HCl pH 7.5

75 μ L 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 μ L 1 M Tris-HCl pH 8

6 μ L 2.5 M CaCl₂

Make up to 5 mL



Prepare lysate for extraction

1 Prepare lysate for extraction

1.1 Prepare 100 μ L lysis buffer per sample.

Lysis solution	99 μ L
RNase solution (10 mg/mL)	1 μ L
Total	100 μ L

The Zymo lysis solution can be bought separately.


This protocol also works with the Zymo Solid Tissue Buffer II that is supplied with the Quick-DNA Magbead Plus kit. Solid Tissue Buffer II comes as a 2x concentrate and has to be diluted with nuclease-free water.

1.2 Dissect e.g. 1 *M. hyperodae* pupa for a single sample.

Place up to 88 individuals in a 1000 μ L, round well deepwell plate


Add 2 3.2 mm stainless steel ball bearings and 100 μ L of lysis buffer with RNase to each well.

1.3 Homogenise the tissue for **90 seconds at 1200 RPM** using a plate-compatible tissue homogenizer, e.g. SPEX SamplePrep 2010 Geno/Grinder.

 00:01:30

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


Incubate at 37°C for 30 minutes.

 00:30:00

 37 °C

1.5 Add 5 μ L Proteinase K solution (20 mg/mL) and mix on the plate shaker.

Incubate at 55°C for 120 minutes, shaking the plate for one minute every 30 minutes.

 02:00:00

🔥 55 °C

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

🌀 1000 rpm, 15°C, 00:10:00

1.7 Transfer 100 µL of lysate to a 0.8 mL deepwell 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: 300 µL MBB : 5 µl beads	30	26 45	50 85	75 25	99 65	124 05	148 45	172 85	197 25	221 65	246 05	270 45
2: DNA pre-wash buffer	100	334 0	534 0	734 0	934 0	113 40	133 40	153 40	173 40	193 40	213 40	233 40
3: g-DNA wash buffer	100	534 0	934 0	133 40	173 40	213 40	253 40	293 40	333 40	373 40	413 40	453 40
4: Tris-HCl	10	702	902	110 2	130 2	150 2	170 2	190 2	210 2	230 2	250 2	270 2

3 Run the epMotion protocol 88x Quick-DNA Magbead Plus Low Volume 40_6.

3.1 **Run the protocol with level sensing enabled.**

