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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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1 Works for me dx.doi.org/10.17504/protocols.io.birqkd5w

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MATERIALS

NAME	CATALOG #	VENDOR
3.2 mm stainless steel beads, RNase free	NEXSSB32-RNA	
96 Well 0.8mL Plate (Bulk)	AB0859	Thermo Fisher
ZymoBIOMICS Lysis Solution	D4300-1-150	Zymo Research
Quick-DNA Magbead Plus Kit	D4082	Zymo Research

MATERIALS TEXT

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 Prepare 100 µL lysis buffer per sample.

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

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 µL, round well deepwell plate

Add 2 3.2 mm stainless steel ball bearings and 100 µL 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 µL 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

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Reagent	Reservoir volume (mL)	8	16	24	32	40	48	56	64	72	80	88
1: 300 µL MBB : 5 µl beads	**30**	**2645**	**5085**	**7525**	**9965**	**12405**	**14845**	**17285**	**19725**	**22165**	**24605**	**27045**
2: DNA pre-wash buffer	100	3340	5340	7340	9340	11340	13340	15340	17340	19340	21340	23340
3: g-DNA wash buffer	100	5340	9340	13340	17340	21340	25340	29340	33340	37340	41340	45340
4: Tris-HCl	10	702	902	1102	1302	1502	1702	1902	2102	2302	2502	2702

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

3.1 ⚠

Run the protocol with level sensing enabled.

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