Version 2

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MagAttract + Metapolyzyme metagenomic gDNA extraction from urine V.2

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Dogstails

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ABSTRACT

A protocol for the metagenomic extraction of bacterial DNA from urine samples (optimised using dog urine), for use in a rapid diagnostics pipeline. At the end of the protocol, the DNA is cleaned up and ready for rapid barcoding (SQK-RBK004) library preparation for nanopore sequencing (or whatever other application you want to do).

Unless otherwise stated, all reagents should be included in the listed kits.

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Coming soon.

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GUIDELINES

This protocol, an adaptation of Qiagen's MagAttract HMW DNA kit, was developed by Natalie Ring and Alison Low for the Dogstails project, a collaboration between the Roslin Institute and the Royal (Dick) School of Veterinary Studies funded by the Dogs Trust. We are grateful to the dogs (and their owners) who donated samples to the R(D)SVS's Hospital for Small Animals, many of which were used in the development of this protocol.

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@NatalieAnneRing

MATERIALS TEXT

Kits

- Urine sample from which to extract metagenomic gDNA

MagAttract HMW DNA kit **Qiagen Catalog #67563**

ProNex Size-Selective Purification System **Promega Catalog #NG2001**

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854** In 2 steps

Other reagents

- 50 mM Tris, 10 mM EDTA, ph8.0 ("buffer P1")

MetaPolyzyme **Sigma Aldrich Catalog #MAC4L-5MG**

- Nuclease-free Water **Contributed by users**
- Distilled Water **Contributed by users**

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Equipment

DNA LoBind tubes, 1.5 mL
Tubes

Eppendorf **022431021**

1.5 mL

OR

SafeSeal reaction tube, 1.5 ml, PP, PCR Performance Tested, Low DNA-binding
Tubes

Sarstedt **72.706.700**

1.5 mL

Magnetic Stand
Magnetic Stand

Thermo Scientific **MR02**

Any magnetic rack that fits your tubes will suffice.

Centrifuge
Benchtop Centrifuge

Eppendorf **5405000441**

Any benchtop centrifuge will suffice

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ThermoMixer
Benchtop Incubator

Eppendorf **5382000023**

Any heat block will suffice

Mini-centrifuge
Centrifuge

Fisher **S67601B**

Any standard mini centrifuge with adapters for different tube sizes will suffice

Vortex Mixer
Vortex Mixer

VWR **97043-562**

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BEFORE STARTING

- **"Buffer P1"** is required for the metapolyzyme lysis incubation: 50 mM Tris, 10 mM EDTA, pH 8.0
- **Metapolyzyme** is used here at a concentration of 3.3 mg/ml (resuspend 5 mg lyophilized powder in 1.5 ml PBS pH 7.5)
- We recommend using **low DNA-binding tubes** throughout, but definitely for the elution/storage of DNA

Extended pre-lysis spin down

1 Pellet 2x 1.5 ml aliquots of urine in 1.5 ml tubes by centrifuging at maximum speed (~13,000 RPM/16,000 xg) for 20 minutes, then discard supernatant 20m

3 mL urine

16,000 x g, Room temperature, 00:20:00

We have found that this extended spin at the beginning of the protocol results in much better yield of bacterial gDNA, especially in samples with low bacterial abundance

Metapolyzyme & Proteinase K Lysis

2 Resuspend cell pellets (which might be invisible) and combine in 160 µl buffer P1 (50 mM Tris, 10 mM EDTA, pH 8.0)

160 µL buffer P1

3 Add 20 µl metapolyzyme (3.3 mg/ml, 5 mg resuspended in 1500 µl PBS) and mix by flicking the tube

20 µL metapolyzyme (3.3 mg/ml)

4 Incubate on a thermomixer for 60 minutes at 37°C with 900 RPM shaking 1h

900 rpm, 37°C, 01:00:00

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5 Add 20 µl MagAttract proteinase K and mix by flicking the tube

20 µL proteinase K

6 Incubate on a thermomixer for 30 minutes at 56°C with 900 RPM shaking 30m

900 rpm, 56°C, 00:30:00

MagAttract DNA isolation and washing

7 Add 150 µl MagAttract buffer AL and mix by pulse vortexing

150 µL buffer AL

Our standard "pulse vortex" is 10 short (<1 second) pulses per tube

8 Add 15 µl MagAttract Suspension G and 280 µl MagAttract buffer MB and mix by pulse vortexing

15 µL Suspension G

280 µL Buffer MB

Make sure the magnetic beads (Suspension G) are really well mixed before adding them! The whole suspension should be black, not separated into a bead layer and a clear layer. We usually resuspended by vortexing for 10 or more seconds.

9 Incubate on a thermomixer for 3 minutes at room temperature with 1,400 RPM shaking

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1400 rpm, Room temperature , 00:03:00

10 Spin down briefly, then pellet beads on magnet and remove supernatant

11 Add 700 µl MagAttract buffer MW1 and incubate on a thermomixer for 1 minute at room temperature with 1,400 RPM shaking 1m

700 µL buffer MW1

1400 rpm, Room temperature , 00:01:00

12 Repeat steps 10 and 11 1m

13 Spin down briefly, then pellet beads on magnet and remove supernatant

14 Add 700 µl MagAttract buffer PE and incubate on a thermomixer for 1 minute at room temperature with 1,400 RPM shaking 1m

700 µL buffer PE

1400 rpm, Room temperature , 00:01:00

15 Repeat steps 13 and 14 1m

16 Spin down briefly, then pellet beads on magnet and remove supernatant

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17 Rinse the pelleted beads on the magnetic rack with 700 µl distilled water by pipetting down the opposite wall of the tube, then incubate for 1 minute on the magnetic rack

700 µL distilled water

18 Remove distilled water

19 Repeat steps 17 and 18

20 Spin down briefly, then pellet beads on magnet and remove any remaining supernatant

21 Add 50 µl nuclease-free water off the magnet, to resuspend the bead pellet

50 µL nuclease-free water

22 Incubate on a thermomixer for 3 minutes at room temperature with 1,400 RPM shaking 3m

1400 rpm, Room temperature , 00:03:00

23 Spin down briefly, then pellet beads on magnetic rack and **keep supernatant** in a low-DNA binding 1.5 mL tube (e.g. Eppendorf or Sarstedt)

Qubit Pre-clean-up quantification

24 Quantify DNA using Qubit dsDNA HS kit. If DNA concentration is an appropriate concentration for your experiment (for us, this means at least 0.2 ng/µl), continue to clean-up steps.

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**

1 µL DNA

199 µL Qubit dsDNA HS working solution

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ProNex DNA clean-up

25 Add 150 µl room temperature ProNex beads to your entire tube of DNA (49 µl)

200 µL ProNex beads

Like the magnetic beads in Suspension G, make sure the ProNex beads are really well mixed (10+ seconds of vortexing) immediately before you use them.

26 Mix well by slowly pipetting up and down 10 times

27 Incubate at room temperature for 10 minutes (no shaking needed) 10m

00:10:00

Room temperature

28 Spin down briefly, then pellet beads on magnet and remove supernatant

29 Rinse the pelleted beads on the magnetic rack by pipetting 200 µl ProNex Wash Buffer down **the opposite wall of the tube**, then incubate at room temperature for 60 seconds (no shaking), then remove Wash Buffer 1m

200 µL Wash Buffer

Room temperature

00:01:00

30 Repeat step 26

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31 **Air-dry** (lid open) the sample on the magnetic rack for 5 minutes (longer is OK, no more than 60 minutes) 5m

Room temperature

00:05:00

32 Add 20 µl nuclease-free water off the magnet. Resuspend the pellet by **flicking the tube**, then incubate at room temperature for 5 minutes (no shaking needed) 5m

20 µL nuclease-free water

Room temperature

00:05:00

33 Spin down briefly, then pellet the beads on magnet and **keep supernatant** in a low DNA-binding tube

Qubit post-clean-up quantification

34 Quantify DNA using Qubit dsDNA HS kit. If DNA concentration is an appropriate concentration for your experiment (for us, this means at least 0.2 ng/µl), continue to library preparation.

Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**

1 µL DNA

199 µL Qubit dsDNA HS working solution

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