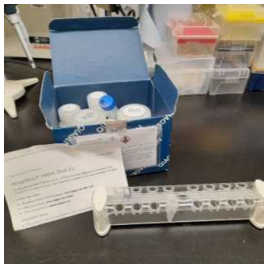
Oct 10, 2022 Version 2

MagAttract + Metapolyzyme metagenomic gDNA extraction from urine V.2



DOI
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Dogstails

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Coming soon.

Protocol status: Working
We use this protocol and it's working

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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.

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.

Please follow on Twitter for latest updates, papers and results:

@NatalieAnneRing

Materials

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

Other reagents

- 50 mM Tris, 10 mM EDTA, ph8.0 ("buffer P1")
- MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**MAC4L-5MG
- Nuclease-free Water
- Distilled Water

Equipment

Equipment	
DNA LoBind tubes, 1.5 mL	NAME
Tubes	TYPE
Eppendorf	BRAND
022431021	SKU
https://online-shop.eppendorf.us/US-en/Laboratory-Consumables-44512/Tubes-44515/DNA-LoBind-Tubes-PF-56252.html	LINK
1.5 mL	SPECIFICATIONS

OR

Equipment

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

NAME

Tubes

TYPE

Sarstedt

BRAND

72.706.700

SKU

https://www.sarstedt.com/en/products/laboratory/screw-cap-micro-tubes-reaction-tubes/reaction-tubes/product/72.706.700/

LINK

1.5 mL

SPECIFICATIONS

Equipment

Magnetic Stand

NAME

Magnetic Stand

TYPE

Thermo Scientific

BRAND

MR02

SKU

https://www.thermofisher.com/order/catalog/product/MR02

LINK

Any magnetic rack that fits your tubes will suffice.

SPECIFICATIONS

Equipment

Centrifuge

NAME

Benchtop Centrifuge

TYPE

Eppendorf

BRAND

5405000441

SKU

https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html

LINK

Any benchtop centrifuge will suffice

SPECIFICATIONS

Equipment

ThermoMixer

NAME

Benchtop Incubator

TYPE

Eppendorf

BRAND

5382000023

SKU

https://online-shop.eppendorf.us/US-en/Temperature-Control-and-Mixing-44518/Instruments-44519/Eppendorf-ThermoMixerC-PF-19703.html

LINK

Any heat block will suffice

SPECIFICATIONS

Equipment

Mini-centrifuge

NAME

Centrifuge

TYPE

Fisher

BRAND

S67601B

SKU

https://www.fishersci.com/shop/products/fisherbrand-standard-mini-centrifuge-standard-mini-centrifuge/s67601b

LINK

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

SPECIFICATIONS

Equipment

Vortex Mixer

NAME

Vortex Mixer

TYPE

VWR

BRAND

97043-562

SKU

https://us.vwr.com/store/catalog/product.jsp?catalog_number=97043-562

LINK

Protocol materials

- Nuclease-free Water
- MagAttract HMW DNA kit **Qiagen** **Catalog #**67563
- Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific** **Catalog #**Q32854
- MetaPolyzyme **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**MAC4L-5MG
- ProNex Size-Selective Purification System **Promega** **Catalog #**NG2001
- Distilled Water

Before start

- **"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 2× 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

Note

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

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

Note

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

Note

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

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

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

ProNex DNA clean-up

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

200 µL ProNex beads

Note

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

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