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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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Version created by Natalie Ring

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

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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

System Promega Catalog #NG2001

Scientific Catalog #Q32854 In 2 steps

Other reagents

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

- Aldrich Catalog #MAC4L-5MG
- Nuclease-free Water Contributed by users
- Ø Distilled Water Contributed by users



Equipment

OR

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

Sarstedt 72.706.700

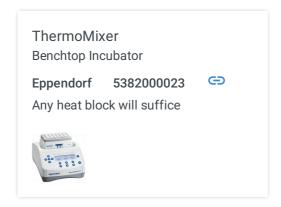
1.5 mL

Magnetic Stand
Magnetic Stand

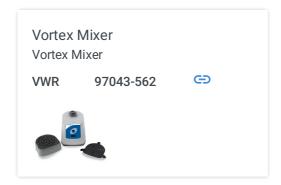
Thermo Scientific MR02

Any magnetic rack that fits your tubes will suffice.









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

- Pellet 2x 1.5 ml aliquots of urine in 1.5 ml tubes by centrifuging at maximum speed (\sim 13,000 RPM/16,000 xg) for 20 minutes, then discard supernatant
 - **■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 μ l 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			
	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 heads (Suspension G) are really well mixed before adding the	ml		

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
 - ■700 µL buffer MW1
 - **\$\price\$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

(🕠 proto	cols.io 8
		■199 µL Qubit dsDNA HS working solution
		□1 µL DNA
	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.
		re-clean-up quantification
	23	Spin down briefly, then pellet beads on magnetic rack and keep supernatant in a low-DNA binding 1.5 mL tube (e.g. <u>Eppendorf</u> or <u>Sarstedt</u>)
		△1400 rpm, Room temperature , 00:03:00
	22	Incubate on a thermomixer for 3 minutes at room temperature with 1,400 RPM shaking
		⊒50 μL nuclease-free water
	21	Add 50 µl nuclease-free water off the magnet, to resuspend the bead pellet
	20	Spin down briefly, then pellet beads on magnet and remove any remaining supernatant
	19	Repeat steps 17 and 18
	18	Remove distilled water
		■700 μL distilled water
	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

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ProNex DNA clean-up

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

■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
- **8** 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
 - ■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)

§ Room temperature

© 00:05:00

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

■20 µL nuclease-free water

8 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.

Scientific Catalog #Q32854

■1 μL DNA

■199 µL Qubit dsDNA HS working solution