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MagAttract + Metapolyzyme metagenomic gDNA extraction from urine

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| 1 | Works for me | Shar | e |
|---|--------------|------|---|
| | | | |

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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.

PROTOCOL CITATION

Natalie Ring 2022. MagAttract + Metapolyzyme metagenomic gDNA extraction from urine. **protocols.io**

https://protocols.io/view/magattract-metapolyzyme-metagenomic-gdna-extractio-cgaxtsfn

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Dogs Trust

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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.

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

@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")

⊠ MetaPolyzyme **Sigma**

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

Equipment



DNA LoBind tubes, 1.5 mL Tubes

Eppendorf 022431021 🖘

1.5 mL

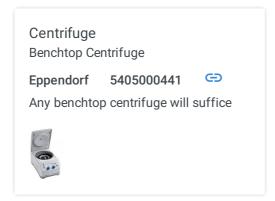
OR

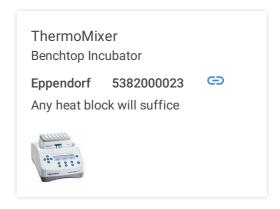
SafeSeal reaction tube, 1.5 ml, PP, PCR Performance Tested, Low DNA-binding Tubes

Sarstedt 72.706.700

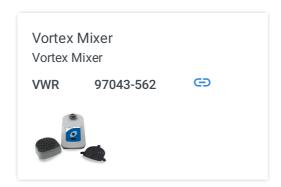
1.5 mL











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 (13,000 RPM) 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 μl buffer P1 (50 mM Tris, 10 mM EDTA, pH 8.0)

■160 µL buffer P1

3 Add 20 μ l metapolyzyme (3.3 mg/ml, 5 mg resuspended in 1500 μ l 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 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 Spin down briefly, then pellet beads on magnet and remove supernatant

| 10 | 10. Add 700 µl MagAttract buffer MW1 and incubate on a thermomixer for 1 minute at room temperature with 1,400 RPM shaking | 1 |
|----|--|---|
| | □700 μL buffer MW1 △1400 rpm, Room temperature , 00:01:00 | |
| | | |
| 11 | 11. Repeat steps 9 and 10 | า |
| 12 | 12. Spin down briefly, then pellet beads on magnet and remove supernatant | |
| 13 | 13. Add 700 µl MagAttract buffer PE and incubate on a thermomixer for 1 minute at room temperature with 1,400 RPM shaking | า |
| | □700 μL buffer PE △1400 rpm, Room temperature , 00:01:00 | |
| 14 | 14. Repeat steps 9 and 10 | า |
| 15 | 15. Spin down briefly, then pellet beads on magnet and remove supernatant | |
| 16 | 16. Rinse the pelleted beads on the magnetic rack with 700 μ l distilled water by pipetting down the opposite wall of the tube | |
| | ⊒700 μL distilled water | |

- 17 17. Repeat step 16
- 18 18. Add 50 µl nuclease-free water off the magnet, to resuspend the bead pellet
 - ■50 µL nuclease-free water
- 19 19. Incubate on a thermomixer for 3 minutes at room temperature with 1,400 RPM shaking
 - **△1400 rpm, Room temperature , 00:03:00**
- 20. 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>)

Qubit Pre-clean-up quantification

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.

Scientific Catalog #Q32854

- ■1 µL DNA
- ■199 µL Qubit dsDNA HS working solution

ProNex DNA clean-up

- 22 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.

| 24 | Incubate at room temperature for 10 minutes (no shaking needed) © 00:10:00 8 Room temperature |
|----|---|
| 25 | Spin down briefly, then pellet beads on magnet and remove supernatant |
| 26 | 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 8 Room temperature 9 00:01:00 |
| 27 | Repeat step 26 |
| 28 | Air-dry (lid open) the sample on the magnetic rack for 5 minutes (longer is OK, no more than 60 minutes) 8 Room temperature 9 00:05:00 |
| 29 | Add 20 µl nuclease-free water off the magnet. Resuspend the pellet by flicking the tube , 5mthen incubate at room temperature for 5 minutes (no shaking needed) 20 µL nuclease-free water 8 Room temperature 9 00:05:00 |

Mix well by slowly pipetting up and down 10 times

23

30 Spin down briefly, then pellet the beads on magnet and keep supernatant in a low DNAbinding tube

Qubit post-clean-up quantification

31 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