



Sep 12, 2022

# MagAttract + Metapolyzyme metagenomic gDNA extraction from urine

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1 Works for me

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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>



## FUNDERS ACKNOWLEDGEMENT

Dogs Trust  
Grant ID: Dogstails

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

Coming soon.

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CREATED

Sep 07, 2022

LAST MODIFIED

Sep 12, 2022

PROTOCOL INTEGER ID

69687

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](#)

[🔗 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](#)

#### 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



ThermoMixer  
Benchtop Incubator

Eppendorf 5382000023 [Link](#)

Any heat block will suffice



Mini-centrifuge  
Centrifuge

Fisher S67601B [Link](#)

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



Vortex Mixer  
Vortex Mixer

VWR 97043-562 [Link](#)



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<sup>20m</sup> 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 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 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<sup>1m</sup> temperature with 1,400 RPM shaking

 **700 µL buffer MW1**

 **1400 rpm, Room temperature , 00:01:00**

11 11. Repeat steps 9 and 10 1m

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<sup>1m</sup> temperature with 1,400 RPM shaking

 **700 µL buffer PE**



 **1400 rpm, Room temperature , 00:01:00**

14 14. Repeat steps 9 and 10 1m

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 µl 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<sup>3m</sup>
-  **1400 rpm, Room temperature , 00:03:00**
- 20 20. 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

- 21 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

- 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.



- 23 Mix well by slowly pipetting up and down 10 times
- 24 Incubate at room temperature for 10 minutes (no shaking needed) 10m
- 🕒 00:10:00
- 🌡 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 <sup>1m</sup>  
**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
- 27 Repeat step 26
- 28 **Air-dry** (lid open) the sample on the magnetic rack for 5 minutes (longer is OK, no more than <sup>5m</sup>  
60 minutes)
- 🌡 Room temperature
- 🕒 00:05:00
- 29 Add 20 µl nuclease-free water off the magnet. Resuspend the pellet by **flicking the tube**, <sup>5m</sup>  
then incubate at room temperature for 5 minutes (no shaking needed)
- 📄 20 µL nuclease-free water
- 🌡 Room temperature
- 🕒 00:05:00

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