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

Manual extraction of High Molecular Weight DNA from single mosquitoes using the Qiagen MagAttract HMW DNA kit

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ABSTRACT

This is a protocol for the manual extraction of high molecular weight (HMW) DNA from insects. It uses the Qiagen MagAttract kit and factors in modifications described in the ChromiumTMGenome Reagent Kits User Guide pages 6-8 (https://support.10xgenomics.com/genome-exome/library-prep/doc/user-guide-chromium-genome-reagent-kit-v2-chemistry). It includes further modifications that benefited two particular needs. First, most reagents are halved per extraction (apart from beads) due to small specimen size (2 mg is a typical weight of an *Anopheles* mosquito, which is far less than the 25 mg of tissue the MagAttract kit can support). Second, due to this small specimen size, we need to maximize DNA yield, so we also perform a second elution to release more DNA.

DNA resulting from this protocol can be further sheared and cleaned for successful PacBio HiFi sequencing. In our experience, a single fresh *Anopheles gambiae* mosquito weighing 2-3 mg yields about 600-800 ng of DNA (quantified using qubit HS DNA kit) using this protocol. Following shearing using G-tubes or the MegaRuptor and SPRI based clean up, we typically retain about 200 ng of sheared DNA, which is just about sufficient to reach 25x coverage PacBio HiFi of a 250 Mb genome. The quality and quantity of DNA is best when starting with a snap frozen from living specimen. However, we have also successfully extracted HMW DNA using this protocol from ethanol and DESS preserved specimens held at room temperature for as long as a week, as long as the specimen was punctured or gently squished to ensure rapid penetration of the preservative. See: *Squishing insects for preservation of HMW DNA in the field* https://www.protocols.io/view/squishing-insects-for-preservation-of-hmw-dna-in-t-cyp3xvqn

We normally perform up to 8 DNA extractions in parallel (this will also depend on which magnetic rack is being used). Please add a comment to let the wider community know if it has worked or not on your species.

NB -- an automated version of this protocol that works on the Kingfisher Apex is also available here on protocols.io

GUIDELINES

This protocol is an adaptation of Chromium™Genome Reagent Kits User guide and Qiagen's MagAttract® HMW DNA Handbook. The Chromium protocol suggests the use of PBS at the beginning. Also, both SOPs recommend using a thermomixer for wash steps, but we find doing this by hand works fine. Finally, we use 1.5 mL microcentrifuge tubes because the plastic pestles that we use to grind insect tissue do not support effective grinding in the 2 mL microcentrifuge tubes recommended by both original protocols. There are, however, pestles available to use in 2 mL microcentrifuge tubes.

MATERIALS

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

Ethanol absolute **Merck Millipore (EMD Millipore) Catalog #107017**

1X PBS (Phosphate-buffered saline)

Dry Ice **Contributed by users**

Equipment

ThermoMixer® C

NAME

Eppendorf

BRAND

5382000031

SKU

https://www.eppendorf.com/gb-en/eShop-Products/Temperature-Control-and-Mixing/Instruments/Eppendorf-ThermoMixerC-p-PF-19703?gclid=Cj0KCQjwn9CgBhDjARIsAD15h0CNOlZBUgxtlqb7IFhPATCy01lk4tpBsMrqDjU7eNcDDSjLBbTjArkaAsb7EALw_wcB&gclsrc=aw.ds

LINK

Equipment

Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey

NAME

minicentrifuge

TYPE

Fisherbrand™

BRAND

16617645

SKU

https://www.fishersci.co.uk/shop/products/fisherbrand-standard-mini-centrifuge/16617645

LINK

Equipment

DNA LoBind® Tubes	NAME
microcentrifuge tubes	TYPE
Eppendorf	BRAND
0030108051	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Laboratory-Consumables/Tubes/DNA-LoBind-Tubes-p-0030108051	LINK

Equipment

Pestle for 1.5 mL Microtube, 100/pk	NAME
pellet pestle	TYPE
Cole-Parmer Essentials	BRAND
WZ-44468-19	SKU
https://www.coleparmer.co.uk/i/cole-parmer-essentials-pestle-for-1-5-ml-microtube-100-pk/4446819	LINK

Equipment

1,000 µl graduated TipOne® Filter Tip, Natural, Racks (sterile), Case

NAME

Pipette tips

TYPE

Starlab

BRAND

S1126-7810

SKU

https://www.starlabgroup.com/GB-en/product/1000-ul-graduated-tipone-filter-tip-natural-sterile-pf-sl-920414.html?childSku=S1126-7810

LINK

Equipment

200 µl Filter Tip / Wide Orifice

NAME

Pipette tips

TYPE

Starlab

BRAND

E1011-8618

SKU

https://www.starlabgroup.com/GB-en/product/200-ul-filter-tip--wide-orifice-e1011-8618.html

LINK

Equipment

DynaMag™-2 Magnet	NAME
Magnetic tube rack	TYPE
Invitrogen™	BRAND
12321D	SKU
https://www.thermofisher.com/order/catalog/product/12321D#:~:text=The%20DynaMag%E2%84%A2%2D2%20magnet%20combines%20a%20strong%20magnetic%20attraction,microcentrifuge%20tubes%20in%20numbered%20spaces	LINK

PROTOCOL MATERIALS

Ethanol absolute **Merck Millipore (EMD Millipore) Catalog #107017** Materials

Dry Ice **Contributed by users** Materials

RNase A **Qiagen Catalog #67563** Step 2

Buffer AL **Qiagen Catalog #67563** Step 2

Proteinase K **Contributed by users Catalog #67563** Step 2

gDNA 165kb Analysis Kit 275 Samples **Agilent Technologies Catalog #FP-1002-0275**

Step 31

Buffer PE **Qiagen Catalog #67563** In 2 steps

Buffer MB **Contributed by users Catalog #67563** Step 10

Buffer AE **Qiagen Catalog #19077** Step 29

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

Step 31

MagAttract Suspension G **Qiagen Catalog #67563** Step 9

Buffer AE **Qiagen Catalog #67563** In 2 steps

Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog #P11496**

Step 31

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

Buffer MW1 **Contributed by users Catalog #67563** Step 13

Nuclease-Free Water **Qiagen Catalog #67563** Step 23

SAFETY WARNINGS

Buffers AL, MB, and MW 1 contain guanidine hydrochloride/guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample preparation waste. Waste needs to be collected in a suitable vessel and disposed of in accordance with local regulations.

BEFORE START INSTRUCTIONS

Ensure all surfaces have been cleaned with 70-80% Ethanol (and ideally bleach before that). Have cleaning wipes for forceps. All kit components, buffers, and RNase A stock solution can be stored at room temperature (15–25°C) for up to 1 year. The box should be labelled with received date. Mix Buffer AL thoroughly by shaking before use. Buffers MW1 and PE are supplied as a concentrate. Before using for the first time, be sure to add the appropriate amount of ethanol (96–100%) as indicated on the bottle. Many components of the kit are also available from Qiagen separately.

Procedure

2h 24m

1 Prepare an open insulated box of dry ice to store sample tubes on whilst working through steps 2-4.

2 Make mastermix of reagents for lysis.

Note

Make sure to choose the right size of tube for preparing the mastermix.

Calculate the mastermix volumes for the number of samples plus 1 for spare pipetting volume.

Volumes per sample are: 100 µL 1X PBS (Phosphate-buffered saline); 10 µL Proteinase K **Contributed by users Catalog #67563** (Mix by inverting the tube 5 times); 2 µL RNase A **Qiagen Catalog #67563** ; 75 µL Buffer AL **Qiagen Catalog #67563** (mix by inversion)

3 For each sample, add 187 µL of the mastermix from step 2 into a new 1.5 mL DNA LoBind tube.

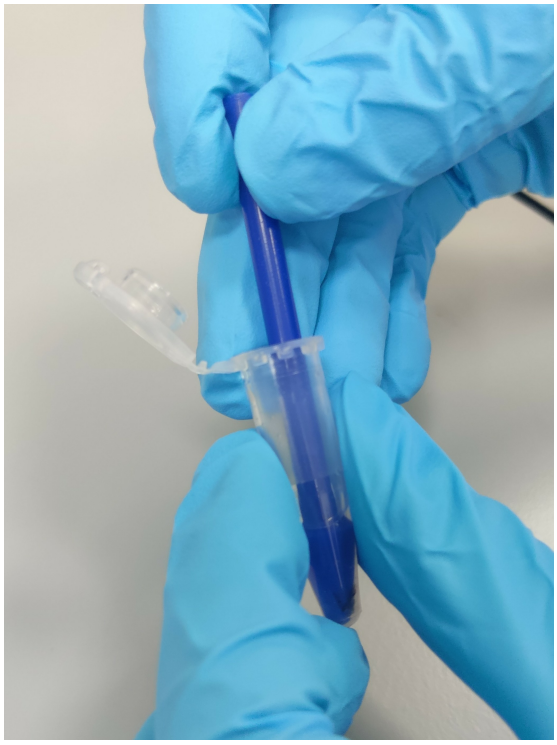
Note

A 2 mL DNA LoBind tube can also be used but an appropriate size pestle will have to be used.

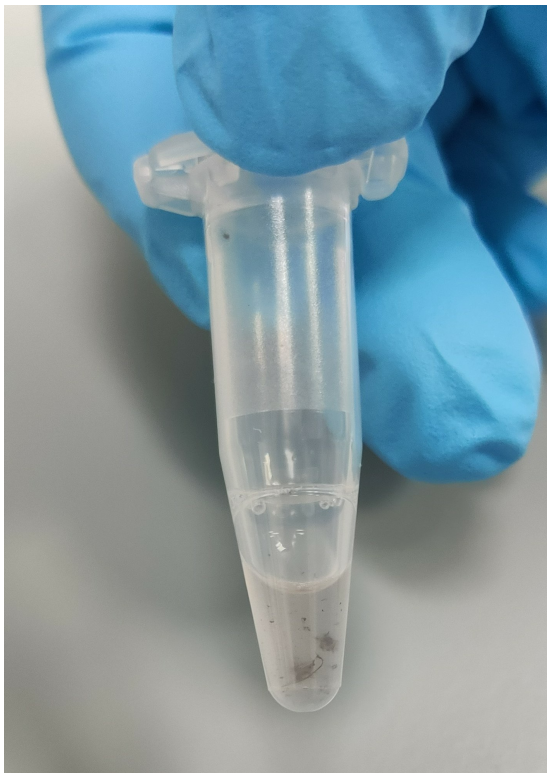
4 Carefully remove the Sample from the sample tube using clean forceps. If the Sample has been stored in a preservation liquid, lightly make contact on a clean piece of tissue to remove surface liquid from the sample. Submerge the Sample into the mastermix in a tube (see step 3) with clean forceps. Insert a sterile pestle in the tube and smash, smear, squash, twist, grind the tissue against the wall of the tube for 00:01:00. There should be no recognisable body parts visible following pestle smashing, only flakes. Place the sample in a tube rack on the bench. Clean forceps with 100% ethanol.

1m

Mosquito in lysis buffer.



Tissue disruption with an autoclavable pestle.



Mosquito debris after tissue disruption.

5 Repeat step 4 for the remaining samples.

6 Briefly spin all samples in a minicentrifuge or similar to collect solution at bottom before next step.

7 Incubate the sample at 25 °C for 02:00:00. 2h

8 Briefly spin samples to collect solution at the bottom of the tube.

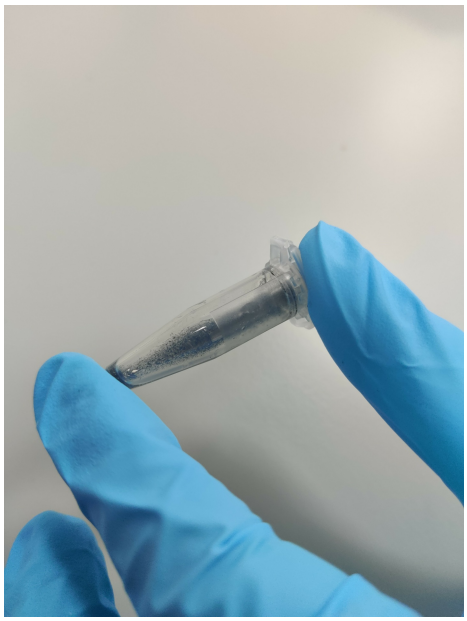
9 Vortex the MagAttract Suspension G Qiagen Catalog #67563 for 00:01:00 and add 15 µL to each sample. 1m

Note

If this is the first time using MagAttract Suspension G Qiagen Catalog #67563, increase the vortexing time to 00:03:00. Briefly vortex MagAttract Suspension G Qiagen Catalog #67563 before adding to each subsequent sample.

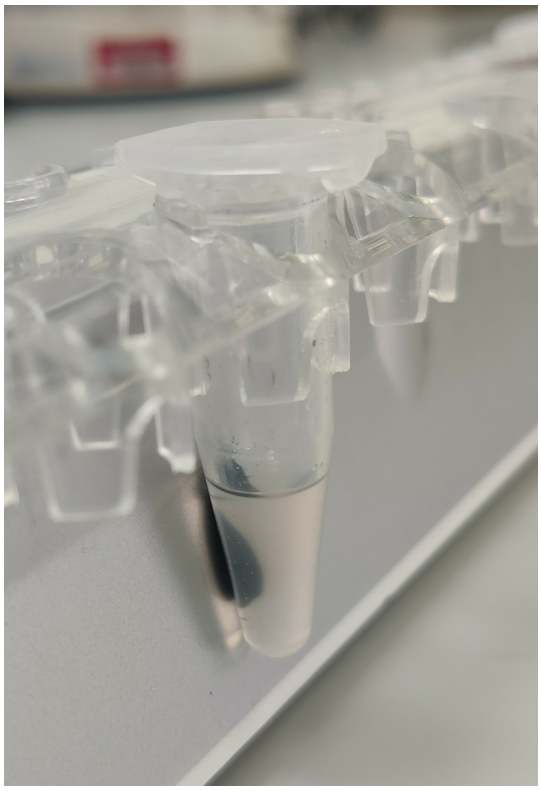
10 Add 140 µL Buffer MB Contributed by users Catalog #67563 to each sample. Mix by gentle inversion, fully invert but don't shake. If you see the beads making flakey clumps, a little like gold leaf, this is a good sign. This is difficult to do simultaneously as you want to see the mixing. If you are doing 8 samples, give all samples another gentle inversion after the last one. 1m

Leave at least 00:01:00 for the beads to bind. Doing multiple samples will often take more time than this.



Flakes of beads building in the lysis buffer after addition of buffer MB and careful inverting of tube.

11 Centrifuge the tube briefly and place on a DynaMag™-2 Magnetic Rack for 00:01:00 to allow bead capture. 1m

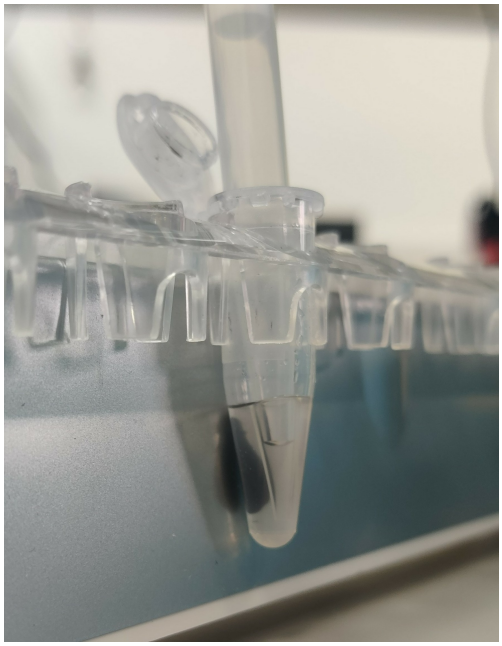


Beads are collecting on the magnetic side.

Note

We use a DynaMag^{TM}-2 Magnetic Rack but other magnetic racks suitable for 1.5 or 2 mL microcentrifuge tubes will work as well.

12 Remove and discard the supernatant. Take care not to disturb the bead pellet.



Supernatant being removed without disturbing the pellet.

13 Remove the Sample from the magnetic rack. Add 350 µL Buffer MW1 **Contributed by users Catalog #67563** directly to the bead pellet. Mix by inversion, ensuring that the beads have come away from the side of the tube. This often requires tapping the tube or swilling the contents. Try to be as gentle as possible. Again, this is difficult to do simultaneously as you need to check each sample.

14 Centrifuge the tube briefly and place on a DynaMagTM-2 Magnetic Rack for 00:01:00 to allow bead capture. 1m

15 Remove and discard the supernatant. Take care not to disturb the bead pellet.

16 Repeat steps 13-15 for a total of 2 washes.

17 Remove the sample from the magnetic rack. Add 350 µL Buffer PE Qiagen Catalog #67563 directly to the bead pellet. Mix by inversion, ensuring that the beads have come away from the side of the tube. This often requires tapping the tube or swilling the contents. Try to be as gentle as possible. Again, this is difficult to do simultaneously as you need to check each sample.

18 Centrifuge the tube briefly and place on a DynaMagTM-2 Magnetic Rack for 00:01:00 to allow bead capture. 1m

19 Remove and discard the supernatant. Take care not to disturb the bead pellet.

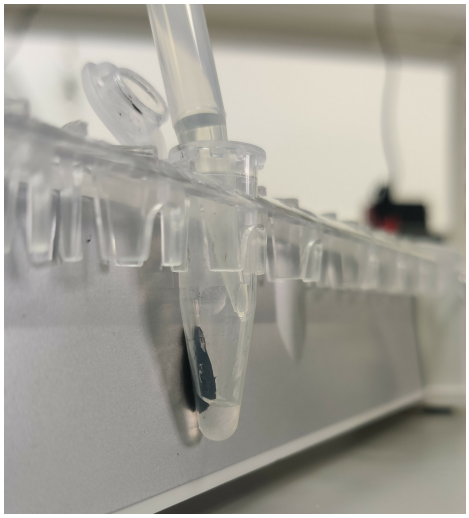
20 Repeat steps 17 and 18 for a total of 2 washes but do not remove supernatant immediately, proceed to step 21.

21 If you have more than four tubes, split them into groups of four or fewer. Perform steps 22-24 (water wash) on the first group of samples, whilst the remaining samples wait in Buffer PE Qiagen Catalog #67563 on the magnet. [While the first group of samples are eluting in step 25-26 below, it is possible to perform the water wash on the second group of samples.]

22 Remove and discard the supernatant. Take care not to disturb the bead pellet. With a P20 pipette remove any remaining supernatant. Leave the sample on the magnetic rack for the next step. Do not pipette water directly onto the beads.

Note

The timing of the next step is extremely important. If a multichannel pipette is not available, ensure that each tube has the exact same incubation time. Do not exceed 00:01:00.



Water being pipetted against the side opposite of the magnetic beads to avoid disturbing beads.

23 Carefully add 350 µL Nuclease-Free Water **Qiagen Catalog #67563** down the side of the tube opposite the magnetic pellet. Start a timer counting up from zero. After 00:00:15 add water to the second sample, after 00:00:30 add to the third sample, after 00:00:45 add to the fourth sample. At 00:01:00 remove and discard the water from the first sample, at 00:01:15 remove and discard water from the second sample, at 00:01:30 the third, and 00:01:45 the fourth. This will enable multiple samples to be incubated for exactly 00:01:00. 8m

24 Repeat step 23 for a total of 2 washes.

25 Remove the samples from the magnetic rack. Add 100 µL Buffer AE **Qiagen Catalog #67563** directly to the bead pellet of each sample. Ensure that the pellet is submerged and has come away from the side of the tube. Incubate at 25 °C and 1400 rpm shaking for 00:03:00. 3m

26 During this 00:03:00 incubation perform steps 22-25 on the second group of samples if present. 3m

27 Centrifuge each tube briefly and place them on a magnetic rack for 00:01:00 to allow bead capture. 1m

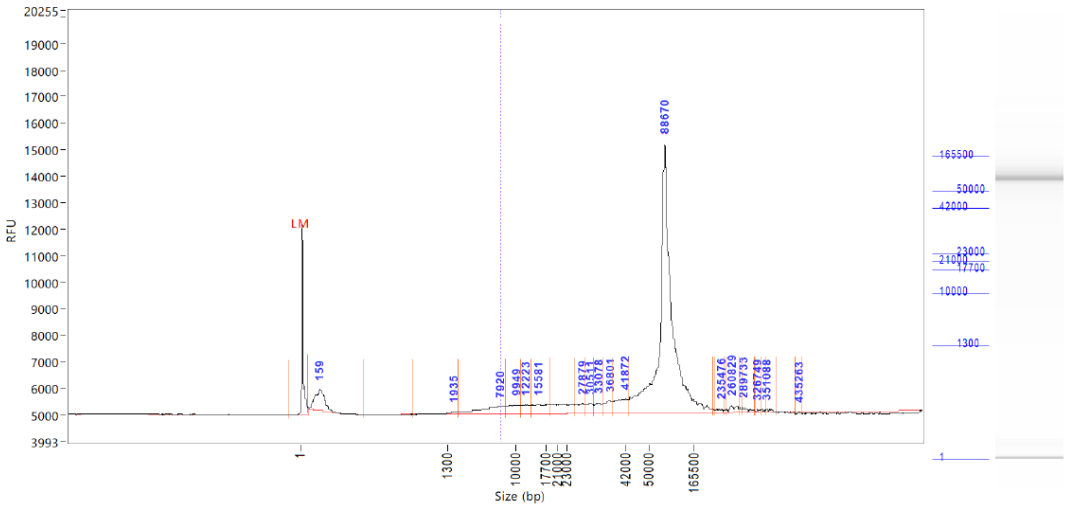
28 Using a wide-orifice pipette tip, carefully transfer the supernatant containing purified gDNA to a new labelled 1.5 mL LoBind microcentrifuge tube or barcoded (e.g. FluidX) tube.

29 Second elution: remove the samples from the magnetic rack. Add 100 µL Buffer AE **Qiagen Catalog #67563** directly to the bead pellet of each sample. Ensure that the pellet is submerged and has come away from the side of the tube. Incubate at 25 °C and 1400 rpm shaking for 00:03:00. 3m

> Note
>
> Due to the second elution step, an additional bottle of Buffer AE **Qiagen Catalog #19077** will be necessary if you buy the kit.

30 Using a wide-orifice pipette tip, carefully transfer the supernatant containing purified gDNA to the same 1.5 ml LoBind microcentrifuge tube or Fluidx tube with a final volume of 200 µL.

31 Store the extracted gDNA sample at 4 °C. Assess the quantity of DNA extracted using the Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854** or Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog #P11496** and assess the quality of the DNA using the Femto Pulse gDNA 165kb Analysis Kit 275 Samples **Agilent Technologies Catalog #FP-1002-0275**.



Example of a Femtopulse profile of DNA extracted from a single snap frozen *Anopheles* mosquito with this protocol.