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Automated high throughput Qiagen MagAttract High Molecular Weight DNA extraction from mosquitoes

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Fiona Teltscher¹, Mara Lawniczak¹

¹Wellcome Sanger Institute



Fiona Teltscher

Wellcome Sanger Institute

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Protocol status: Working

We use this protocol and it's working

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Keywords: magattract, high molecular weight DNA, hmw DNA extraction, kingfisher, high-throughput, dna from insect, extracted hmw dna, dna yield, sheared dna, single anopheles mosquito, chromiumtmgenome reagent kits user guide, quantity of dna, ng of dna, hmw dna, more dna, mb genome, protocol for the extraction, dna, qiagen magattract kit, mosquito, high molecular weight, extraction, preserved specimen, small specimen size

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Abstract

This is an automated protocol for the 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>). Due to small specimen size, we need to maximize DNA yield. so we also do a double 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 *Anopheles* mosquito preserved using best practices (snap frozen from living) weighing 2-3 mg yields about 600-800 ng of DNA using this protocol. Following shearing using g-tubes (Covaris) 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 100% ethanol and DESS (DMSO salt solution) preserved specimens held at room temperature for as long as a week, as long as the specimen was gently squished to ensure rapid penetration of the preservative.

This protocol has been used successfully on a wide range of arthropod species as part of the Tree of Life Programme at the Wellcome Sanger Institute. Please use the commenting function to share whether this protocol worked or not for your species. If you make modifications to improve this protocol so it works better for your species, please use the forking function in protocols.io to share these adjustments.

Materials

 MagAttract_HMW_Lawniczak_King... 2.9KB

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

 KingFisher™ Plastics for 96 deep-well format **Thermo Fisher Scientific Catalog #95040450B**

Equipment	
KingFisher™ Plastics for 96 deep-well format	NAME
KingFisher 96 Deep-well Plate, Barcoded	TYPE
Thermo Scientific™	BRAND
95040450B	SKU
https://www.thermofisher.com/order/catalog/product/95040450B	LINK

Equipment	
KingFisher™ Plastics for 96 deep-well format	NAME
KingFisher 96 tip comb for deep-well magnets	TYPE
Thermo Scientific™	BRAND
97002534	SKU
https://www.thermofisher.com/order/catalog/product/97002534	LINK

Equipment

KingFisher Apex Benchtop Sample Prep	NAME
Nucleic Acid Purification System	TYPE
Thermo Scientific™	BRAND
5400930	SKU
https://www.thermofisher.com/order/catalog/product/5400930	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	
Multipette® E3	NAME
multi-dispenser pipette	TYPE
Eppendorf	BRAND
4987000371	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Liquid-Handling/Manual-Pipettes-Dispensers/MultipetteE3-MultipetteE3x-p-4987000371	LINK

Equipment

Combitips® advanced

NAME

multi-dispenser pipette tips

TYPE

Eppendorf

BRAND

various sizes

SKU

<https://www.eppendorf.com/gb-en/eShop-Products/Liquid-Handling/Pipette-Tips/Combitips-advanced-p-PF-18250>

LIN
K

Equipment

micropestles

NAME

micropestly

TYPE

Eppendorf

BRAND

EP0030120973

SKU

https://www.sigmaaldrich.com/GB/en/product/sigma/ep0030120973?srsItd=AfmBOopyrmPB1JHqmZcVYE12gf_bdlbKWySiH8Lx_7FiTMF_CK7gdYpu

LIN
K



Protocol materials

- ✕ MagAttract HMW DNA kit **Qiagen Catalog #67563**
- ✕ gDNA 165kb Analysis Kit 275 Samples **Agilent Technologies Catalog #FP-1002-0275**
- ✕ Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**
- ✕ Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog #P11496**
- ✕ Buffer AL **Qiagen Catalog #67563**
- ✕ 1X PBS (Phosphate-buffered saline)
- ✕ Proteinase K **Qiagen Catalog #67563**
- ✕ RNase A **Qiagen Catalog #67563**
- ✕ KingFisher™ Plastics for 96 deep-well format **Thermo Fisher Scientific Catalog #95040450B**
- ✕ Nuclease-Free Water **Qiagen Catalog #129114**
- ✕ Buffer PE **Qiagen Catalog #19065**
- ✕ MagAttract Suspension G **Qiagen Catalog #67563**
- ✕ Buffer MB **Catalog #67563**
- ✕ Buffer AE **Qiagen Catalog #19077**
- ✕ Buffer MW1 **Catalog #67563**
- ✕ Tape Pads **Qiagen Catalog #19570**

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 local regulations.

Before start

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.



Sample preparation

2h 1m

- 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: 200 μ L

1X PBS (Phosphate-buffered saline) ; 20 μ L

Proteinase K **Qiagen Catalog #67563** (Mix by inverting the tube 5 times);

4 μ L RNase A **Qiagen Catalog #67563** and 150 μ L

Buffer AL **Qiagen Catalog #67563** (mix by inversion)

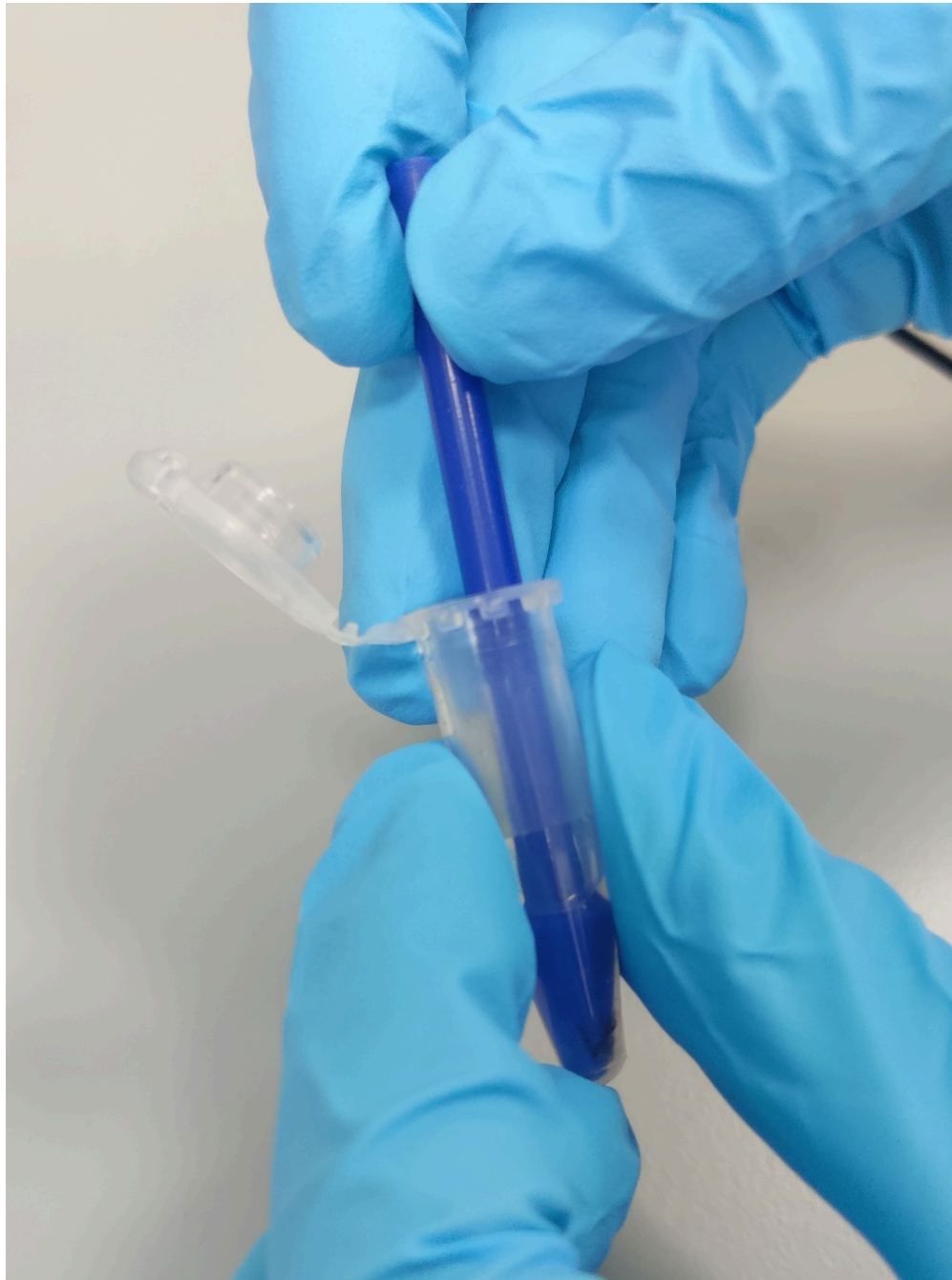
- 3 For each sample, add 374 μ L of the mastermix from step 2 into a new 1.5 ml DNA LoBind tube.

- 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 tubes (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 70-100% ethanol.

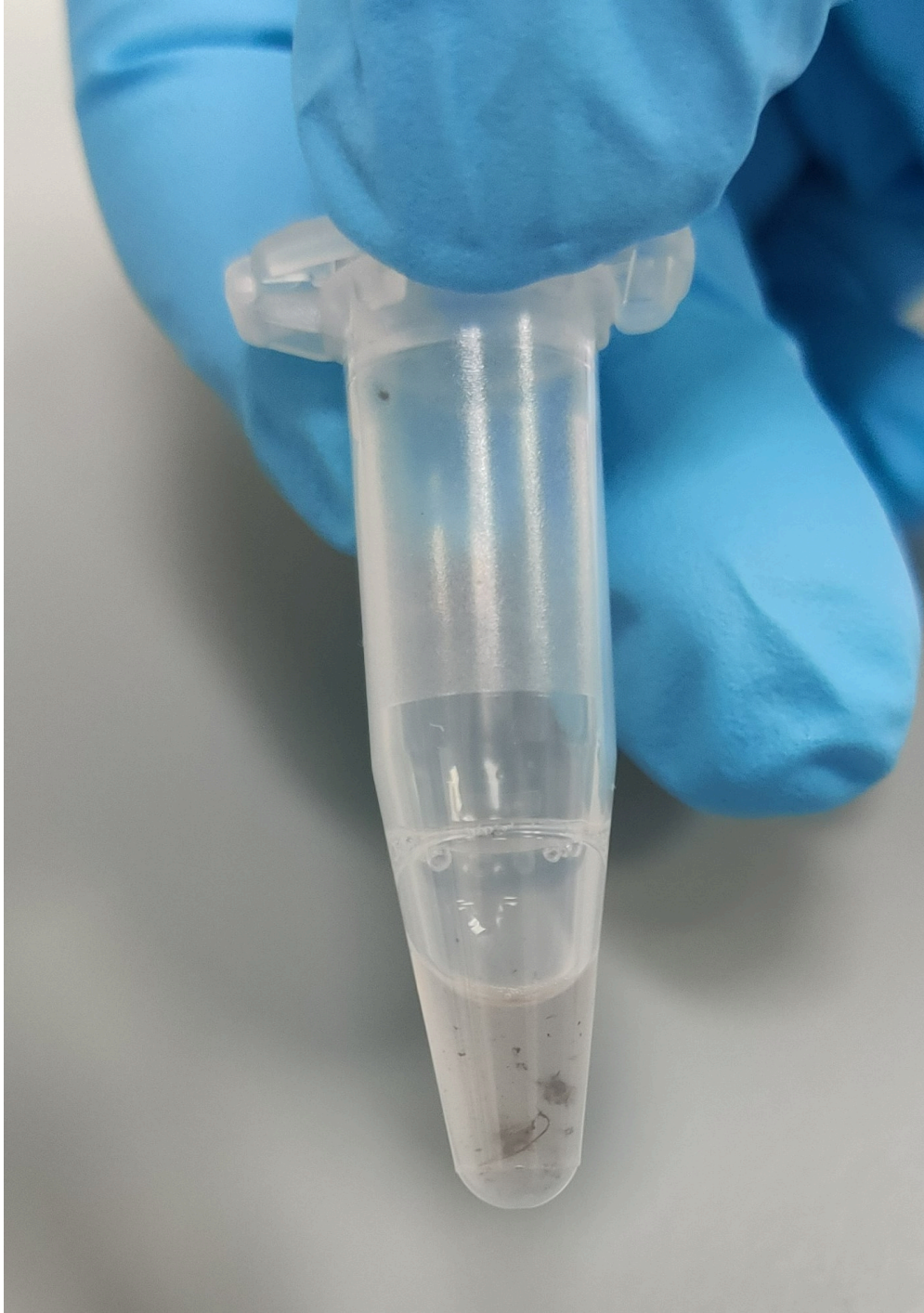
1m



Mosquito in tissue 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 (or room temperature if no heat block is available) for

2h

 02:00:00

Preparation of plates for the Kingfisher Apex

2h 50m

8 Unpack 9 Kingfisher Deepwell 96 well plates

Note

Different plates are available - barcoded and not barcoded, "sterile" (individually wrapped) and standard (wrapped as a set of 5).

Note

While the samples are incubating for two hours, the plates for the Kingfisher Apex can be prepared. We either cover them with clean wipes or proceed to load them onto the Kingfisher to avoid contamination.

9 Prepare wash buffers included in the

 MagAttract HMW DNA kit **Qiagen Catalog #67563** by adding Ethanol to them.

10 Prepare and fill the plates with  Buffer AE **Qiagen Catalog #19077** ,

 Nuclease-Free Water **Qiagen Catalog #129114** ,

 Buffer PE **Qiagen Catalog #19065** ,  Buffer MW1 **Catalog #67563** ,

 MagAttract Suspension G **Qiagen Catalog #67563** and

 Buffer MB **Catalog #67563** as suggested below:

	A	B	C	D	E
	Plate type	Catalog number	Label	Content	Volume
	KingFisher 96 deep-well plate and 96 tip comb for deep-well magnets	95040450 and 97002534	Magattract tip plate, date and initials	Plate with tip comb	N/A



A	B	C	D	E
KingFisher 96 deep-well plate	95040450	Elution 2, date and initials	Buffer AE	200µl
KingFisher 96 deep-well plate	95040450	Elution 1, date and initials	Buffer AE	200µl
KingFisher 96 deep-well plate	95040450	NFW Wash, date and initials	Nuclease-free water	500µl
KingFisher 96 deep-well plate	95040450	PE Wash 2, date, initials	Buffer PE	700µl
KingFisher 96 deep-well plate	95040450	PE Wash 1, date, initials	Buffer PE	700µl
KingFisher 96 deep-well plate	95040450	MW1 Wash 2, date, initials	Buffer MW1	700µl
KingFisher 96 deep-well plate	95040450	MW1 Wash 1, date, initials	Buffer MW1	700µl
KingFisher 96 deep-well plate	95040450	Sample Plate, date, initials	Suspension G magnetic beads and Buffer MB	15µl (Suspension G) and 280µl (Buffer MB)

Note

Kingfisher 96 deep-well plates are available barcoded (B suffix to the catalog number) and not barcoded and packed as a pack of 5 (95040450) and individually wrapped (95040460), either of them will work. The barcode reader inside the machine will time out after about 10 seconds at which point the machine will prompt the user to either try again or move to the next plate.

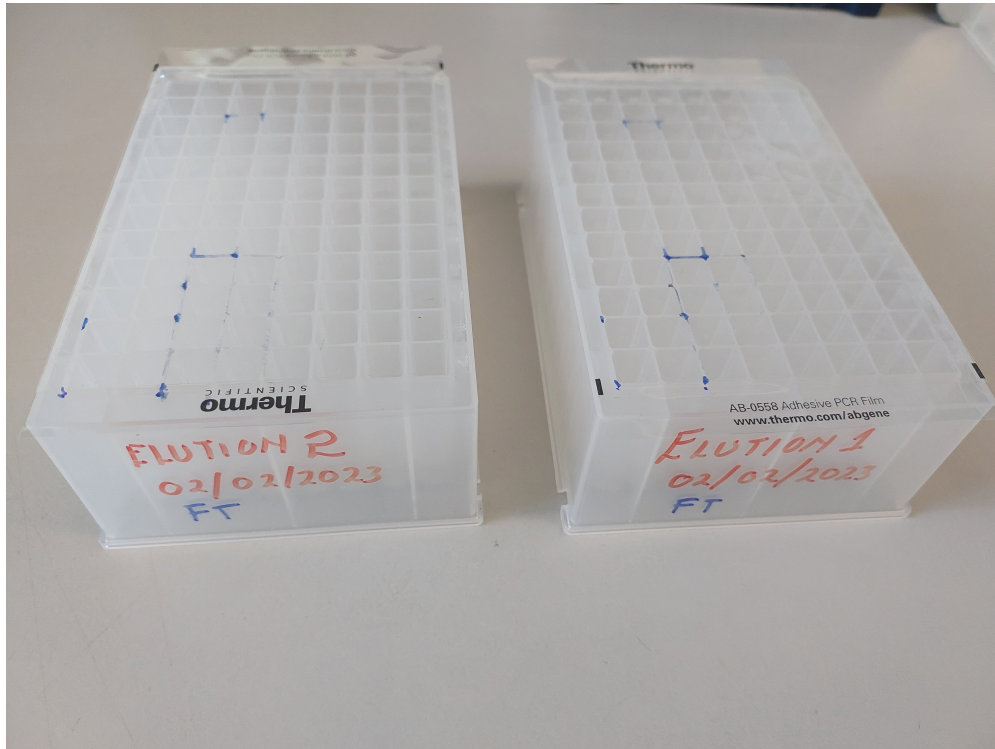
Note

The type of tip comb depends on what magnet head your instrument has. This protocol will work with either the 96 deep-well head or the 96 combi head, but has only been extensively tested with the deep-well head.

Only fill the wells that you intend to use, but the same well will have to be used for each plate. E.g. you start with a new plate and have 4 samples which you intend to run in wells A1, A2, A3 and A4, so you fill those wells in every plate.

Note

A repeater pipette or multichannel pipette can be helpful when a large number of samples is processed.



Plates should be labelled on the opposite side to the letters to make loading them correctly easier. Marking which wells have been used makes reusing them easier and ensures that wells are not accidentally used again.

- 11 Download the programme onto a computer and transfer it to your KingFisher Apex:



MagAttract_HMW_Lawniczak_King...



Equipment

KingFisher Apex System

NAME

Automated DNA purification system

TYPE

ThermoFisher Scientific

BRAND

5400930

SKU

<https://www.thermofisher.com/order/catalog/product/5400930>^{LINK}

- 12 Start your KingFisher Apex and select the correct protocol to run.

Note

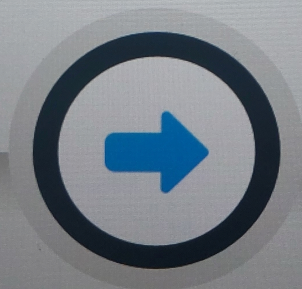
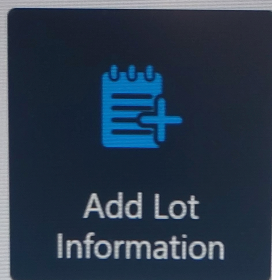
We recommend 1 hour of UV light either before or after every run to avoid contamination between runs.

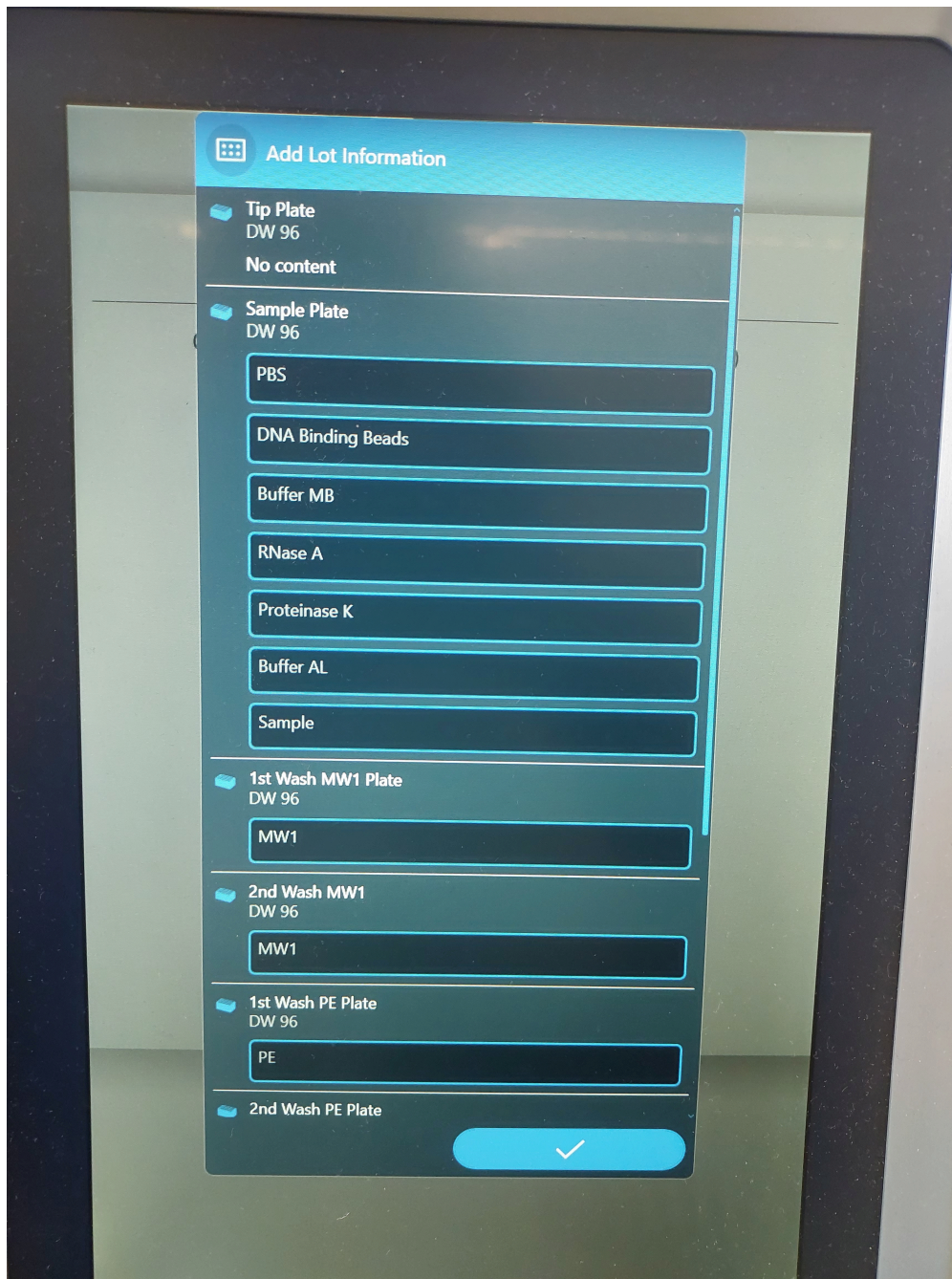
Note

When starting and choosing the programme make sure you have the correct magnet head attached. In this protocol we are using the 96 deep well head and the corresponding tip comb

- 13 The software allows you to input batch/lot information for each reagent. This is optional but highly recommended.

Click below to add lot information (Optional)





- 14 Load all but the sample plate onto the instrument.
- 15 Once the  02:00:00 incubation time for the sample has elapsed add the sample to the sample plate using wide-orifice pipette tips. Make sure to keep a record which sample was added in which well.

2h

- 16 Load the sample plate including the sample onto the instrument and start the programme. It takes about 🕒 00:50:00 .

50m





Post-extraction

- 17 Once the KingFisher Apex is finished the plates can be removed as prompted. Elution 1 and 2 for each sample can be combined into the same tube (making sure to use wide-orifice tips).

Note

Be careful to combine elution 1 and 2 of the same sample and to not mix up elution 1 and 2 of different samples at this step.

Used plates that still have unused wells can be reused in future runs. Either carefully tip out the waste without contaminating unused wells or keep buffers in.

Note

If you keep the buffer in the wells make sure to reuse the plates soon after as some of the buffers can crystallise and cause the tip comb to not fit into the well anymore, leading to an error message and a potentially failed run.

Mark which wells were used and cover plates with a film, such as

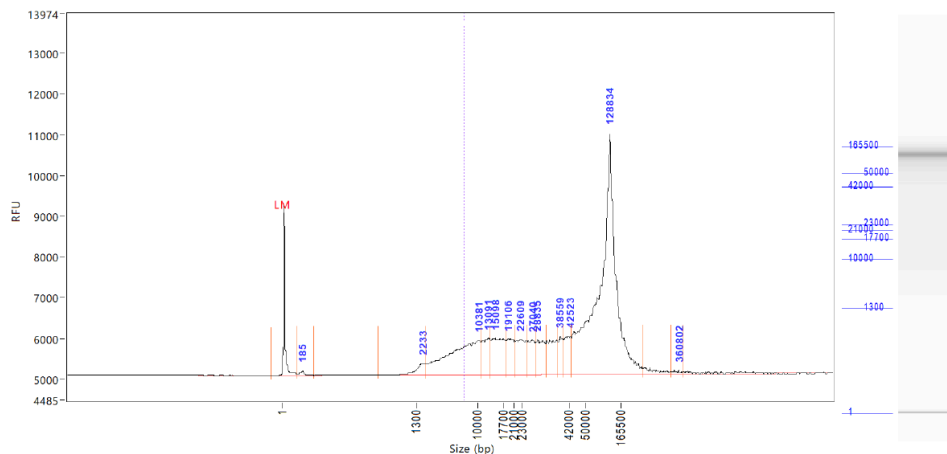
 Tape Pads **Qiagen Catalog #19570** . When reusing plates that still have used buffers from previous runs in some wells make sure you only run the same programme with the same volume to avoid spillage in the machine.

18 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





Protocol references

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>).

Qiagen MagAttract Manual

<https://www.qiagen.com/gb/resources/resourcedetail?id=3e331b6c-0742-483b-b74f-a7be2d4c1116&lang=en>

Edel Sheerin, Filipa Sampaio, graeme oatley, Maja Todorovic, Michelle Strickland, Raquel Juliana Vionette do Amaral, Caroline Howard 2023. Sanger Tree of Life HMW DNA Extraction: Automated MagAttract v.1. **protocols.io**<https://dx.doi.org/10.17504/protocols.io.x54v9p2z1g3e/v1>