

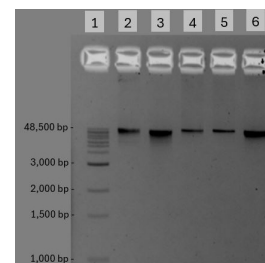
Sep 01, 2025

Version 1

Efficient extraction of high molecular weight dsDNA from bacteriophage (QIAGEN silica-based membrane method) V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5k2m6v1b/v1>



Mitchell G Hedges¹, Samuel Montgomery²

¹The Kids Research Institute Australia; ²The Kids Research Institute Australia (formerly Telethon Kids Institute)



Mitchell Hedges

The Kids Research Institute Australia

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5k2m6v1b/v1>

Protocol Citation: Mitchell G Hedges, Samuel Montgomery 2025. Efficient extraction of high molecular weight dsDNA from bacteriophage (QIAGEN silica-based membrane method). **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.8epv5k2m6v1b/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: September 01, 2025

Last Modified: September 01, 2025

Protocol Integer ID: 226129

Keywords: dsDNA, DNA, bacteriophage, phage, DNEasy, Blood & Tissue, QIAGEN, silica-based membrane, silica, column, high, molecular, weight, extraction, DNA extraction, PhageWA, Oxford Nanopore, Nanopore, sequencing, whole genome sequencing, WGS, ONT, genome, fast, 2 hours, dsdna bacteriophage, efficient extraction of high molecular weight dsdna, phage dna concentration, phage dna, yield of phage dna, high molecular weight dsdna, high molecular weight dna, bacteriophage, impact from host cell dna contamination, host cell dna contamination, phage, read whole genome sequencing, phage lysate, oxford nanopore technology, whole genome sequencing, such as oxford nanopore technology, such as the qiagen dneasy blood, qiagen dneasy blood

Funders Acknowledgements:

Medical Research Future Fund

Grant ID: 2023559

Disclaimer

The digestion step (where exogenous bacterial nucleic acids are degraded) may require optimisation i.e. longer incubation times or additional volume of enzyme. The selected volume of 5uL of DNase and RNase has been tested and successfully applied in our phage extractions, and has been shown to minimise the amount of host DNA reporting via whole genome sequencing.

Sufficient removal of bacterial nucleic acids can be dependent on the growth media used for bacterial propagation, the growth characteristics of the host, the composition of the phage collection buffer and ultimately the yield of bacterial nucleic acid liberated during phage propagation.

Note that our group has detected nucleic acids within commercially available propagation broths ranging between ~2 - 10 ng/uL of DNA and ~25 - 100 ng/uL of RNA with fragment sizes from ~50 bp to 200 bp, which are consistently removed following our pre-treatment steps. We note this to alert users that sources of nucleic acid within your sample could be from either host, phage or reagent(s). We recommend the testing of all reagents for endogenous nucleic acids prior to use in DNA extractions.

Abstract

High molecular weight DNA can be efficiently extracted from dsDNA bacteriophages (phage) using a silica-based membrane method, such as the QIAGEN DNeasy Blood & Tissue Kit, with some modifications to significantly reduce the impact from host cell DNA contamination. Users can expect phage DNA concentrations of 2 to 100 ng/uL in 40 uL of elution buffer (median of approximately 15 ng/uL) if extracted from 450 uL of phage lysate containing $> 1 \times 10^9$ PFU/mL. The concentration and yield of phage DNA is appropriate for long read whole genome sequencing, such as Oxford Nanopore Technology.



Image Attribution

Mitchell G Hedges 2025 (20250429_MH_EXPMH143)

Guidelines

- **Expected input:** It is ideal to extract DNA from phages which have a titre of 1×10^9 to 1×10^{11} PFU/mL, however lower titres may be extracted successfully.
- **Expected output:** Users can expect phage DNA concentrations in the range of 2 to 100 ng/uL in 40 uL of elution buffer (median of approximately 15 ng/uL), if extracting from 450 uL of phage lysate containing $> 1 \times 10^9$ PFU/mL.
- **Intended End Use:** Phage DNA extracted using this protocol is appropriate for whole genome sequencing such as Oxford Nanopore Technology long read sequencing, or PCR based applications.

Materials

- DNeasy Blood & Tissue Kit (QIAGEN #69504 or 69506)
- 100% Ethyl alcohol Molecular Biology Grade (Sigma Merck #E7023-1L)
- RNase A, DNase & Protease-Free (Thermo Fisher #EN0531)
- DNase I [2000U/mL] (New England Biolabs #M0303 S/L)
- DNase I 10x reaction buffer (New England Biolabs #B0303SVIAL)
- UltraPure 0.5M EDTA, pH 8.0 (Thermo Fisher #15575020)
- 1.5 ml Eppendorf DNA LoBind tubes (ThermoFisher #30108.051)
- Thermoshaker heating block (Thermo Fisher #0006286)
- Microcentrifuge (Thermo Fisher #43231565)

Before start

This protocol extracts DNA from a 450 μ L aliquot of bacteriophage lysate, however the starting lysate volume can be scaled up. If scaling up, adjust the reagent volumes in all steps until the lysate mixture has been passed through the silica-membrane, ensuring consistent ratios throughout the protocol to this point. Resume the protocol volumes used at the Buffer AW1 step.



Preparation of reagents

5m

1 DNase & RNase Digestion Master Mix

5m

1. Calculate the required volume of Digestion Mix for the number of samples you wish to process
2. Each 450 μL sample of bacteriophage lysate requires 50 μL of Digestion Mix
3. Remove DNase I, DNase 10x buffer and RNase A from storage and place On ice to thaw
4. Gently pipette mix each tube 5 to 10 times. **Do not vortex DNase I as it is sensitive to physical denaturation**
5. To a clean microcentrifuge tube, add 5 μL of DNase I and 5 μL of RNase A to 40 μL of 10x DNase I reaction buffer, store On ice until required

2 DNEasy Blood & Tissue Kit Buffer Preparation

Prepare kits buffers Buffer AW1 and Buffer AW2 with 100% molecular grade ethanol as per the manufacturer's manual

Phage DNA extraction

1h 41m

- 3 Add 50 μL of Digestion Master Mix to 450 μL of syringe filtered 0.22 μm bacteriophage lysate in a 1.5mL LoBind microtube and mix via gentle pipetting
- 4 Incubate for 00:30:00 at 37 $^{\circ}\text{C}$ using a Thermomixer without shaking
Note: longer incubation times may be necessary to achieve complete degradation of bacterial DNA and RNA. It is advisable to quantify the amount of bacterial DNA and RNA pre- and post-digestion to ensure bacterial nucleic acids do not contaminate phage DNA. The volume of enzyme prescribed significantly degrades host DNA and RNA and has been demonstrated to facilitate whole genome sequencing without host DNA contamination. Assessing the DNA quality of both untreated lysate and treated lysate using agarose gel electrophoresis can be useful to determine the host nucleic acid sizes in your samples, and whether a post-extraction SPRI bead clean up may be required (described elsewhere).
- 5 Add 25 μL of 0.5 Mass Percent EDTA (final concentration >20 mM) to inactivate the DNase and incubate for 00:01:00 at 37 $^{\circ}\text{C}$ after gently pipette mixing



- 6 Incubate for 80 °C for 00:15:00 to heat inactivate DNase. Chill the tube at 4 °C for 00:01:00 and spin down tubes briefly to collect condensation. Hot tubes may pop open, so take care to avoid cross contamination 18m
- 7 Add 20 µL proteinase K and 180 µL Buffer ATL to the sample, and mix by vortexing, incubate for 00:15:00 at 56 °C . Total volume of sample is now 720 µL 15m
- 8 Vortex the tube for 15 seconds and spin down tubes 1m
- 9 Aliquot 360 µL of the mixture into a second microcentrifuge tube (ensure it is labelled) to split the sample which allows processing of the volumes in subsequent steps 1m
- 10 To each tube containing the lysis mixture, add 360 µL of Buffer AL and mix thoroughly by vortexing 1m
- 11 Add 360 µL of ethanol (96 – 100%) and mix again thoroughly by vortexing
It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution 1m
- 12 Incubate at 56 °C for 00:05:00 5m
- 13 Pipette 600 µL of the phage lysate mixture into a labelled silica-membrane spin column 1m
- 14 Centrifuge the viral spin column in the collection tube at 6000 x g for 00:01:00 , discarding the throughflow into a waste container. The throughflow is **not compatible with bleach**. Discard the tube to avoid liquid cross contamination 2m
- 15 Repeat the above two steps until all phage sample has passed through the column 6m
- 16 Place the silica-membrane spin column in a new collection tube, discard any used collection tubes and any throughflow 1m
- 17 Add 500 µL of ethanol Wash Buffer 1 (AW1) onto the spin column 1m



- 18 Centrifuge the spin column in the collection tube at 6000 x g for 00:01:00 1m
- 19 Place the spin column in a new and clean collection tube. Discard the throughflow and **do not combine with bleach**. Discard the used collection tube 1m
- 20 Add 500 µL of Wash Buffer 2 (AW2) into the spin column 1m
- 21 Centrifuge the spin column in the collection tube at maximum speed 16000 x g to 20000 x g for 00:01:00 2m
- 22 Discard the throughflow (this is compatible with bleach if required by laboratory processes) and replace the column back into the collection tube and spin at maximum speed 16000 x g to 20000 x g for 00:03:00 to dry the column 4m
- 23 Place the spin column in a labelled, clean 1.5 ml LoBind tube (which will become the final sample tube for storage) and pipette 40 µL of warmed (56 °C) Buffer AE directly onto the membrane. Incubate at 56 °C for 00:01:00 and then centrifuge for 00:01:00 at $\geq$ 6000 x g to elute 3m
- 24 Re-elute the sample by pipetting the eluate back onto the silica membrane and incubate at 56 °C for 00:01:00 then centrifuge for 00:01:00 at $\geq$ 6000 x g to re-elute 2m
- 25 Store the eluate containing the phage DNA at 2 - 8 °C in a labelled tube and take forward to DNA QA/QC processes / whole genome sequencing 1m
- 25.1 **Optional (recommended):** Quantify the extracted DNA using a 1x dsDNA High Sensitivity Kit and a sample volume of 2 µL . Following favourable quantification, load between 5 ng to 20 ng of phage DNA with a loading dye onto a 1% TAE agarose gel prepared with 1X SYBR Safe Stain and run at 100V for 01:00:00 to assess DNA quality. Ensure a ladder such as 1kb Extend DNA Ladder (NEB) is used and loaded at the same mass as the phage DNA sample for visualisation on a trans-UV system such as ChemiDoc



Protocol references

QIAGEN DNeasy Blood & Tissue Handbook (June 2023)

Jakočiūnė D, Moodley A. A Rapid Bacteriophage DNA Extraction Method. *Methods and Protocols*. 2018; 1(3):27.

<https://doi.org/10.3390/mps1030027>