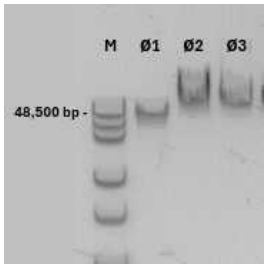
Apr 23, 2025 Version 2

# Extraction of high molecular weight DNA from bacteriophage V.2




Version 1 is forked from Extraction of high molecular weight DNA from nasal lining fluid

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https://dx.doi.org/10.17504/protocols.io.261ge59ddg47/v2

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

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**Keywords:** phage, bacteriophage, DNA extraction, DNA isolation, precipitation, zinc chloride, high molecular weight, HMW DNA, phage concentration, extraction for long read sequencing, DNA, Nanopore, ONT, PureGene, dsDNA, bacteriophage extraction of high molecular weight dna, extraction of high molecular weight dna, bacteriophage extraction, dsdna bacteriophage, high molecular weight dna, bacteriophage virion precipitation, bacteriophage, zinc chloride for bacteriophage virion precipitation, read whole genome sequencing, oxford nanopore technology, dsdna, oxford nanopore, extraction, nanopore, whole genome sequencing, such as oxford nanopore technology, phage, genome

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## Disclaimer

The digestion step (where exogenous bacterial nucleic acids are degraded) may require optimisation i.e. longer incubation times or additional volume of enzyme. 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

Extraction of High Molecular Weight DNA from dsDNA bacteriophages (phage) can be achieved using an isopropanol precipitation method which can be used for long read whole genome sequencing, such as Oxford Nanopore Technology.

This is a forked protocol from https://dx.doi.org/10.17504/protocols.io.3byl4qwqovo5/v1, which instead uses zinc chloride for bacteriophage virion precipitation to concentrate the sample.

## Guidelines

This protocol can be used to extract DNA from bacteriophage within bacterial lysates.
It is ideal to extract phages which have a titre of $1 \times 10^{10}$ to $1 \times 10^{11}$ PFU/mL, however lower titres can be extracted successfully.

## Materials

The following materials were utilised for this protocol:

- Puregene tissue kit (Qiagen, #158023) containing cell lysis solution, protein precipitation buffer, proteinase K, RNase A (4 mg/mL), and DNA hydration buffer
- Ethanol, molecular grade (Sigma, #E7023)
- UltraPure molecular grade water (ThermoFisher, #10977015)
- DNAse I (NEB, #M0303S)
- DNAse I 10x reaction buffer (NEB, #B0303SVIAL)
- Isopropanol, molecular grade (Sigma, #I9516)
- Zinc chloride ($ZnCl_2$) powder (Merck, Cat#: Z0152-50G)
- Hydrochloric acid 32% AR Grade (Chem Supply, Cat# HA020-500M)
- DNA LoBind 2.0mL tubes (Thermo Fisher, #30108.078)

## Safety warnings

While this protocol does not utilise common hazardous chemicals used for DNA extraction (phenol/chloroform), care should be taken to follow the recommendations in the MSDS provided which each reagent, and ensure proper storage for the dangerous goods utilised in this protocol (flammable liquids etc)

## Before start

This protocol extracts DNA from a 700µL aliquot of bacteriophage lysate, however the starting lysate volume can be scaled up. If scaling up, adjust the reagent volumes in all steps up until the zinc chloride pelleting step, ensuring consistent ratios throughout the protocol to this point. Once the zinc chloride pellet has had the supernatant removed, the protocol volumes resume at the described amount.

## Preparation of reagents

17m

1 **Ethanol (70% v/v)** 2m

Create a [M] 70 % (v/v) solution of molecular grade ethanol using UltraPure water (approx. 400 µL per sample)

2 **Digestion Master Mix** 3m

1. Calculate the required volume of Digestion Mix for the number of samples you wish to process
2. Each 700 µL sample of bacteriophage lysate requires 78 µL of Digestion Mix
3. Create this by adding 1 µL (up to 5 uL*) of DNAse I and 1 µL (up to 5 uL*) of RNAse A to 76 µL (adjust to 78 uL final volume) of 10X DNAse I reaction buffer and place on ice.

- The amount of each enzyme can be increased if the lysate has high levels of host DNA or RNA. We recommend quantifying the amount of host nucleic acid in your sample both pre- and post-digestion to understand your specific requirements. Our group has used 5 uL of each enzyme with favourable results.
- Quantify the nucleic acid within untreated and treated samples using a highly sensitive method such as the QuBit HS assay kit(s).

3 **Zinc chloride solution (** [M] 2 Molarity (M) **)** 10m

1. Weigh 0.68 g of $ZnCl_2$ and add to 2.5 mL of molecular grade water
2. Carefully add 2.5 µL of [M] 32 % (v/v) hydrochloric acid to the zinc solution and gently swirl to allow complete dissolution. **Note**: this step generates heat
3. Measure a small aliquot of the solution on a pH meter, a [M] 2 Mass Percent solution of zinc chloride should have a pH pH 4.5 to pH 7 to ensure $Zn^{+2}$ ions are available in solution

4 **EDTA Solution (** [M] 0.1 Mass Percent **)** 2m

Add 200 µL of [M] 0.5 Mass Percent molecular grade EDTA to 800 µL of UltraPure water

## DNA extraction

2h 8m

5 Add 78 µL of Digestion Master Mix to 700 µL of syringe filtered ( 0.22 µm ) bacteriophage lysate in a 1.5mL LoBind microtube and mix via gentle pipetting 1m

6 Incubate for 00:30:00 at 37 °C using a Thermomixer without shaking 30m

**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. Alternatively, increase the volume of DNase I and RNase A within the Digestion Master Mix as discussed above. 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).

7 Add [M] 2.5 % (v/v) of [M] 2 Mass Percent zinc chloride solution (freshly made) to the bacteriophage lysate for a final concentration of [M] 50 millimolar (mM) and mix via gentle pipetting 1m

8 Incubate the sample for 00:05:00 at 37 °C 5m

9 Centrifuge for 00:10:00 at 8000 rcf, Room temperature and remove supernatant by aspiration 10m

10 Add 50 µL of [M] 0.1 Mass Percent EDTA solution to the phage pellet, pipetting and stirring gently with the pipette tip to break up the pellet, noting it is not necessary to resuspend the pellet during this step 1m

11 Add 1.5 µL of Proteinase K ( [M] 20 mg/mL ) and 250 µL of Cell Lysis Solution to the phage pellet and mix by gently pipetting up and down 25 times 1m

12 Incubate for 01:00:00 at 56 °C in a thermomixer at 500 rpm and then pre-cool a centrifuge to 4 °C 1h

13 Place sample on ice for 00:01:00 1m

14 Add 100 µL of protein precipitation solution and mix thoroughly by inverting the tube 50 times 2m

15 Incubate samples on ice for 00:05:00 5m

16 Centrifuge tube at 16000 rcf, 4°C, 00:10:00 10m

16.1 The precipitated proteins should form a tight pellet. If the protein pellet is not tight, or there is still some in solution, repeat the incubation and centrifugation

17 Add supernatant to a new 1.5mL LoBind tube, careful not to disturb the protein pellet 1m

## DNA precipitation 1h 46m

18 Add 300 µL of isopropanol to a new 1.5mL LoBind tube and carefully add the supernatant from the previous step by pipetting from the air-liquid interface the sample, mix by inverting the tube 50 times 3m

19 Incubate at Room temperature for 00:05:00 5m

20 Centrifuge at 16000 rcf, Room temperature, 00:05:00 placing the hinge of the tube at the top of the centrifuge to assist in identifying the location of the DNA pellet once centrifuged 5m

21 Discard the supernatant by slowly drawing with a pipette at the air-liquid interface to avoid disturbing the pellet, which may be difficult to see 1m

> Note
>
> Pellets when precipitated with isopropanol will be near invisible, and easily detached from the tube, so care must be taken not to discard the DNA pellet.

22 Add 300 µL of [M] 70 % (v/v) ethanol to the sample and invert 10 times to wash the pellet 2m

23 Centrifuge at 16000 x g for 00:01:00 1m

24 Discard the supernatant, and allow the pellet to air dry for 00:05:00 5m

25 Add 30 µL of DNA hydration solution or nuclease free water to the pellet 1m

26 Incubate at 65 °C for 01:00:00 to dissolve the DNA, or incubate in the fridge at 4 °C Overnight 1h

27 After this step, DNA is ready to be quantified and used downstream. DNA resuspended in water should be stored at -20 °C, while DNA resuspended in DNA hydration solution can be stored for up to one month at 4 °C

## Protocol references

Saladié M, Caparrós-Martín JA, Agudelo-Romero P, Wark PAB, Stick SM, O'Gara F. Microbiomic Analysis on Low Abundant Respiratory Biomass Samples; Improved Recovery of Microbial DNA From Bronchoalveolar Lavage Fluid. Front Microbiol. 2020 Oct 6;11:572504. doi: 10.3389/fmicb.2020.572504. PMID: 33123104