Feb 13, 2026

Phenol/Chloroform DNA Extraction Protocol with SDS Lysis Buffer

DOI
https://dx.doi.org/10.17504/protocols.io.36wgq1ej5vk5/v1

Mai-Britt Mosbech[1]

[1]Department of Immunology, Genetics and Pathology, SciLifeLab Genomics NGI, Uppsala Genome Center, Uppsala University, Sweden

Biodiversity Genomics E...

Tuuli Lundbäck-Larva
Department of Immunology, Genetics and Pathology, SciLifeLab...

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.36wgq1ej5vk5/v1

Protocol Citation: Mai-Britt Mosbech 2026. Phenol/Chloroform DNA Extraction Protocol with SDS Lysis Buffer. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq1ej5vk5/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: February 04, 2026

Last Modified: February 13, 2026

Protocol Integer ID: 242618

Keywords: BGE, hmw dna extraction, phenol chloroform extraction, sds lysis buffer, pci, dna extraction, Biodiversity Genomics Europe, chloroform hmw dna extraction protocol, chloroform dna extraction protocol with sds lysis buffer, chloroform dna extraction protocol, hmw dna, sds lysis buffer, extraction protocol, phenol, extraction, dna, protocol

Funders Acknowledgements:
Biodiversity Genomics Europe receives funding from the European Union's Horizon Europe Research and Innovation Action
Grant ID: 101059492

Disclaimer

The duration times presented are estimations only.

Abstract

This is a Phenol/Chloroform HMW DNA Extraction Protocol with SDS Lysis Buffer.

Acronyms:
HMW = High molecular weight
SDS = Sodium Dodecyl Sulfate

Attachments

DOCX

PCI_Extraction_SDS_I...

27KB

Materials

- N-lauryl sarcosine
- SDS
- Tris pH 7-9
- EDTA
- Proteinase K
- BME (β-Mercaptoethanol) (optional)
- Nuclease-free water/Milli-Q water
- PVP10 (optional)
- PVP40 (optional)
- PVP360 (optional)
- NaCl
- TE 100x (only if using TE-buffer)
- 2 ml Eppendorf safe-lock tube
- Puregen Proteinase K (2.5 mg/ml final concentration)
- Scalpel + other material to slice tissue, e.g. weighing boat
- Puregen Proteinase K
- Thermoshaker
- Centrifuge
- RNase A
- 2 ml MaXtract High Density phase lock tube, Qiagen
- Pipettes + standard pipette tips and p1000 wide-bore tip
- Minicentrifuge
- Phenol:Chloroform:Isoamyl Alcohol (25:24:1)
- Chloroform:Isoamyl Alcohol (24:1)
- Tube rotator
- 99% ethanol

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and work in a fume hood to perform the laboratory procedures in this protocol. In addition, chloroform-resistant gloves must be worn, and phenol and chloroform waste bottles should be stored in the fume hood.

Waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

Liquid waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

See best practices, thawing and handling instructions, and safety instructions for all the kit reagents, as well as instructions and safety data sheets for other reagents used in this protocol **before starting**.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Before start

- Proteinase K and RNase A are added individually to samples and should not be added to the lysis buffer stock.
- BME (β-Mercaptoethanol) and PVPs (Polyvinylpyrrolidones) can be added to remove polyphenolic compounds and prevent them from binding to the DNA. It has been indicated that BME and the different PVPs bind different types of polyphenols; however, this has not been explicitly confirmed. You can choose to add one or more of these if you suspect phenolic content in your input material.
- The high salt/low ethanol precipitation will precipitate small amounts of polysaccharides, which would otherwise co-precipitate with the DNA.
- For input material high in polysaccharides, we recommend using **CTAB (Cetrimonium bromide) lysis buffer** instead; however, this does not work with the MaXtract tubes due to the high salt content in the lysis buffer. It is possible to buy phase-gel tubes for high salt samples; however, we have not tested these.
- Aim for 25-200 mg tissue depending on tissue type and expected DNA yield. For PacBio HiFi, we would optimally like 20-30 µg of DNA in 200 µl corresponding to 100-150 ng/µl. If you expect a much lower yield, consider eluting in a lower volume as purity measurement by absorbance becomes more unreliable with concentrations below 10 ng/µl.
- Start at step 8 when cleaning already extracted DNA; bring each sample to 405 µl with sample buffer.

Buffer Recipes:

SDS lysis buffer

	Reagents for SDS lysis buffer	Stock	Final conc.	Vol. for 400 µl	Vol. for 10 ml
	N-lauryl Sarcosine	10%	2%	80 µl	2 ml
	SDS	20%	0.5%	10 µl	250 µl
	Tris pH 7-9	1 M	50 mM	20 µl	500 µl
	EDTA	0.5 M	10 mM	8 µl	200 µl
	Proteinase K (add fresh)	20 mg/ml	2.5 mg/ml	50 µl	Add individually
	BME (optional, add fresh)	100%	1 %	4 µl	100 µl
	PVP10 (optional)	10%	1 %	40 µl	1 ml
	PVP40 (optional)	10%	1 %	40 µl	1 ml
	PVP360 (optional)	5%	1 %	80 µl	2 ml
	MQ-H2O	NA	NA	Up to 400 µl	Up to 10 ml

Recipe for SDS lysis buffer

Note

NaCl will be added after the lysis step.

Buffer A

	Reagents for Buffer A	Stock	Final conc.	Vol. for 1 ml
	Tris pH 7-9	1 M	10 mM	10 µl
	EDTA	0.5 M	2 mM	4 µl
	NaCl	5 M	1 M	200 µl
	MQ-H2O	NA	NA	786 µl

Recipe for Buffer A

LowTE-buffer

	Reagents for LowTE-buffer	Stock	Final conc.	Vol. for 50 ml
	Tris pH 7-9	1 M	10 mM	500 µl
	EDTA	0.5 M	0.1 mM	10 µl
	MQ-H2O	NA	NA	49.4 ml

Recipe for LowTE-buffer

TE-buffer

	Reagents for TE buffer	Stock	Final conc.	Vol. for 400 µl
	TE 100x	Tris 1 M/EDTA 100 mM	Tris 10 mM/EDTA 1 mM	500 µl
	MQ-H2O	NA	NA	49.5 ml

Recipe for TE-buffer

PCI Extraction protocol using SDS lysis buffer

4h 45m

1 Label 2 ml Eppendorf safe-lock tube and fill it with 350 µl of working SDS lysis buffer. 1m

2 Slice frozen tissue sample with scalpel and transfer sliced tissue into tube with SDS lysis buffer. 2m

Note

Work fast to get the tissue into the SDS lysis buffer before thawing too much. If tissue is very soft, an Eppendorf pestle can be used to break up the tissue in the SDS lysis buffer by squishing it against the sides of the tube. NO rotating pestle. Never use TissueRuptor together with SDS lysis buffer.

3 Add 50 µl Puregen Proteinase K (2.5 mg/ml final concentration) per tube.

4 Incubate sample at 50°C for at least 00:30:00 . Shake at 900-1400 rpm for the first 10-15 minutes to improve lysis and reduce fragment size for higher purity. Reduce to 300-450 rpm after the 15 min and incubate until all tissue has lysed. 2h

Note

If the tissue is well sliced, complete lysis can occur in 30 minutes at 50°C. In 2 hours, lysis occurs in most of tubes unless they contained high amount of tissue.

5 Lower temperature to 37°C and add 5 µl RNase A (10 mg/ml, final conc. 120 µg/ml). 1m

6 Incubate at 37°C, 300 rpm for 00:30:00 . 30m

7 Label the 2 ml MaXtract High Density phase lock tube from Qiagen. Spin it for 20-30 seconds at 12-16,000 x g. 1m

8 Shortly spin tube with lysate to remove buffer from cap.

9 Transfer lysate with a p1000 wide-bore tip set to 500 µl to phase lock tube. 1m

Note

If a fat layer is present, try to avoid this while pipetting.

10 Add 100 µl of 5 M NaCl (1 M final concentration).

11 In the fume hood, add 400 µl of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). 1m

Note

Before the actual distribution of phenol:chloroform, fill the tip and empty it 2-3 times to avoid dripping.

12 Close tubes firmly and mix the organic and aqueous phase thoroughly to form a transiently homogeneous suspension. Do not vortex. Tube rotation is okay (e.g., 20-30 rpm for 00:10:00). 11m

13 Spin for 00:10:00 at 16,000 x g. 11m

Note

The original protocol says 1 min, but HMW-DNA may need longer spin time to separate phases properly. It may be hard to retrieve the HMW-DNA closest to the gel as this will follow when you try to pipette the viscous DNA liquid.

14 Carefully add 400 µl of Chloroform:Isoamyl Alcohol (24:1) on top of the aqueous phase and mix thoroughly without breaking the gel-seal to the organic phase. Do not vortex! Tube rotator (20 rpm, 00:10:00) seems to be okay for the samples tested at our lab. 11m

Note

As previously, fill and empty the tip several times before distributing to prevent dropping of chloroform.

15 Spin for 00:10:00 at 16,000 x g. 11m

16 Transfer up to 450 µl of the aqueous phase to a new, labeled 2 ml tube and make a note of the transferred volume. 1m

17 (Optional) Add as much buffer A to the phase lock tube as needed, in order for final sample volume to reach 650 µl and mix thoroughly without breaking the gel-seal to the organic phase. Spin for 2-5 minutes at 12,000 x g and transfer approximately the same amount of the aqueous phase as you added in this step to the previously transferred volume for a total of maximum 650 µl. 10m

18 Add 0.3x vol of 99% ethanol and invert tube 20 times to mix. 2m

Note

This high-salt, low-ethanol mixture precipitates polysaccharides while gDNA remains in solution.

19 Spin tube at maximum speed (at least 10,000 x g) for 00:15:00 . 15m

20 Carefully transfer the supernatant to a new labeled 2 ml tube without disturbing the polysaccharide pellet. 1m

Note

No visible pellet may be seen at this step but always transfer as if a pellet is present.

21 Add 1.7x volume of 99% ethanol (according to transferred sample volume before addition of ethanol in step 19) and invert tube 20 times to mix. 2m

22 Spin tube at maximum speed (at least 10,000 x g) for 00:30:00 . 31m

23 Carefully remove supernatant, do not disturb the pellet. 1m

24 Add 1000 µl of freshly prepared 70% ethanol to remove the excess salt; try to avoid dislodging the pellet. 1m

25 Spin tube at maximum speed (at least 10 g) for 00:05:00 . 6m

26 Carefully remove supernatant, do not disturb the pellet. 1m

27 Repeat steps 24-26. 8m

28 Perform a quick spin to gather the residual ethanol at the bottom of the tube and carefully remove with a P20 tip.

29 Let pellet air dry for 00:05:00 at room temperature, taking care not to over dry. 5m

30 Add 100-200µl resuspension buffer (preferred Elution Buffer/lowTE/TE) and leave at RT with gentle mixing overnight to resuspend. Alternatively, leave at 4°C/RT for 3-4 days to increase homogeneity before performing quality control.

Note

It can take several weeks for some samples to re-hydrate properly. The higher the concentration and integrity, the longer it takes for the sample to become homogeneous.