Short Read Eliminator Kit Family

Handbook v2.0 (07/2019)

Table of Contents

Kit Specifications

Contents

Circulomics Short Read Eliminator XS	
Part Number	**SS-100-121-01**
Number of Samples	**16**
Buffer SRE XS	1 mL
Buffer EB	2 mL

Circulomics Short Read Eliminator	
Part Number	**SS-100-101-01**
Number of Samples	**16**
Buffer SRE	1 mL
Buffer EB	2 mL

Circulomics Short Read Eliminator XL	
Part Number	**SS-100-111-01**
Number of Samples	**16**
Buffer SRE XL	1 mL
Buffer EB	2 mL

Prior to Starting

Create 70% EtOH wash buffer by diluting ethanol (96–100%) with DI water.

Storage

All buffers should be stored at room temperature (18–25 °C).

Product Use

Circulomics Short Read Eliminator Kits are intended for research use only.

Version History

See Circulomics Short Read Eliminator Version History Document (www.circulomics.com/support-sre) for a list of kit and protocol changes.

User Supplied Equipment and Reagent List

Equipment	Model
Centrifuge	Eppendorf 5425
Heat Block (or Water Bath)	Fisher Scientific Isotemp Dry Bath Incubator (11-715-125DQ)
1.5 mL DNA LoBind Microcentrifuge Tubes	Eppendorf (022431021)
200 µL Wide Bore Pipette Tips	USA Scientific (1011-8410)
Ethanol (96–100%)	
DI Water	
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA Quantification	Thermo Qubit 3.0, Qubit dsDNA BR Assay Kit

For All Protocols

- Eppendorf DNA LoBind tubes (Eppendorf #022431021) are recommended for most library preparations.

Introduction

The Circulomics Short Read Eliminator Kit Family (SRE XS, SRE, SRE XL) can be used for rapid high-pass size selection of high molecular weight (HMW) DNA samples. This method can significantly enhance mean read length by progressively depleting short DNA up to 10 kb (SRE XS), 25 kb (SRE), or 40 kb (SRE XL) in length (**Figure 1**). Read length N50 can be increased by up to 10 – 30 kb depending on input sample quality. The kits use a centrifugation procedure similar to standard ethanol precipitation techniques. They have been thoroughly tested on Oxford Nanopore MinION/GridION/PromethION. Protocols for PacBio Sequel applications will be released shortly.

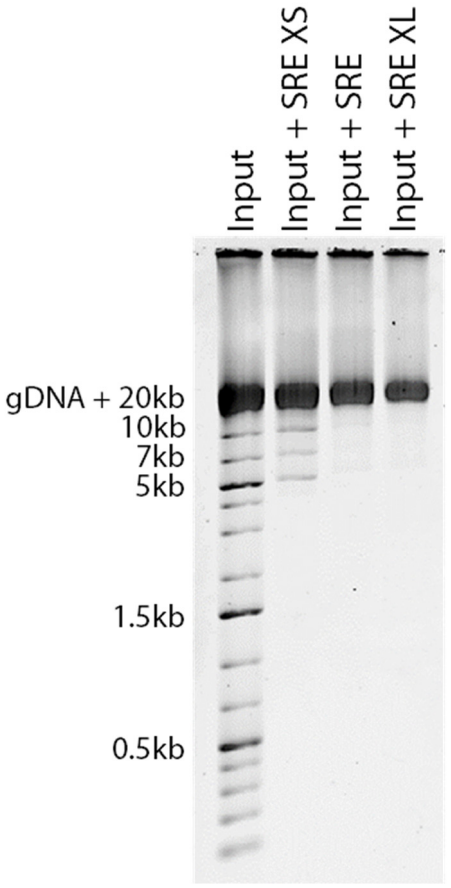


Figure 1. 1% Agarose gel separation of size-selected DNA with size cutoffs demonstrated using a spiked-in ladder (Thermo Scientific GeneRuler 1 kb Plus, #SM1334). Input is 50 ng/µL gDNA extracted from GM12878 cells using Nanobind CBB Big DNA Kit + 20 ng/µL ladder.

Sample Information and Expected Performance

The choice of kit to use should be based on the desired size selection performance and the quality of the input DNA as outlined in the table below. The stated recovery efficiencies will only be achieved when suitable quality input DNA is used at the appropriate Qubit DNA concentration.

Short Read Eliminator Kit Family				
Version	**DNA Depletion Range**	**Qubit DNA Input**	**Recovery Efficiency**	**Sample Notes**
Short Read Eliminator XS	Progressive depletion: <10 kb Near complete depletion: <5 kb	25 – 150 ng/µL	50-90%	Suitable for sheared/fragmented DNA
Short Read Eliminator	Progressive depletion: <25 kb Near complete depletion: <10 kb	50 – 150 ng/µL	50-70%	Requires high quality HMW DNA (>48 kb)
Short Read Eliminator XL	Progressive depletion: <40 kb Near complete depletion: <10 kb	50 – 150 ng/µL	40-50%	Requires very high quality HMW DNA (>>48 kb)

The Short Read Eliminator XS Kit should be used if the DNA sample is sheared/fragmented, has low concentration, or if very high recovery is needed. The Short Read Eliminator Kit requires high quality HMW DNA where the majority of DNA is >48 kb. The Short Read Eliminator XL Kit requires very high quality HMW DNA where the majority of DNA is >> 48 kb. Using a lower quality DNA sample than suggested will result in lower than expected recovery efficiency.

For the Short Read Eliminator XS Kit, the DNA concentration must be between 25 – 150 ng/µL. For the Short Read Eliminator and Short Read Eliminator XL Kits, the DNA concentration must be between 50 – 150 ng/µL. Using a lower than suggested DNA input concentration will result in lower than expected recovery efficiency. Using a higher than suggested DNA input concentration could impact size selection performance.

It is essential that DNA concentration is determined by Qubit or PicoGreen assay. Using concentrations derived solely from UV-Vis measurements will often result in low recovery as the DNA concentration will be over-estimated due to RNA that may also be present in the sample.

The DNA sample should be in TE buffer (pH 8), Circulomics Buffer EB, or water. If the sample buffer differs significantly or contains high levels of salt, the size selection properties and recoveries may be affected.

HMW DNA Size Selection (All Versions)

Each Short Read Eliminator Kit progressively depletes short DNA beginning at its upper cutoff, with depletion efficiency increasing as the DNA gets shorter. **Figure 2** illustrates the performance of the Short Read Eliminator Kit. DNA <10 kb in length is removed to levels that typically cannot be detected by gel or CE. DNA from 10 – 25 kb in length appears progressively removed.

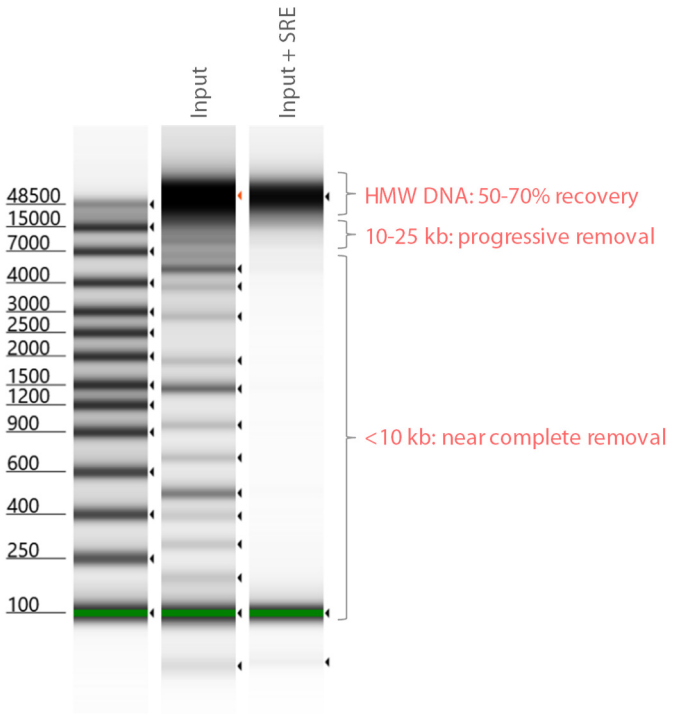


Figure 2. The Short Read Eliminator Kit was used to size select HMW DNA from GM12878 cells that was isolated using the Nanobind CBB Big DNA Kit and spiked with a 1 kb DNA ladder.

The range for near complete depletion is estimated based on agarose gel and TapeStation analysis of HMW DNA samples spiked with ladders as shown in **Figures 1 – 3**. Due to the progressive depletion, size selection is not always apparent when samples containing DNA smears are analyzed by gel or CE. The upper cutoff for progressive depletion is estimated based on comparison of read length distributions between size selected and non-size selected samples as shown in **Figure 5**.

The overall recovery efficiency is highly dependent on the quality and concentration of the input DNA. The Short Read Eliminator XS Kit has the highest overall recoveries and is the most tolerant of sheared/fragmented DNA or low input concentrations. The Short Read Eliminator and Short Read Eliminator XL Kits require high quality HMW DNA. If the samples contain large amounts of fragmented DNA or if the DNA is not sufficiently HMW, the recovery efficiency will be lower than expected. **Figure 3** shows the expected recovery efficiency for Nanobind extracted HMW gDNA samples that were size selected using each of the Short Read Eliminator Kits.

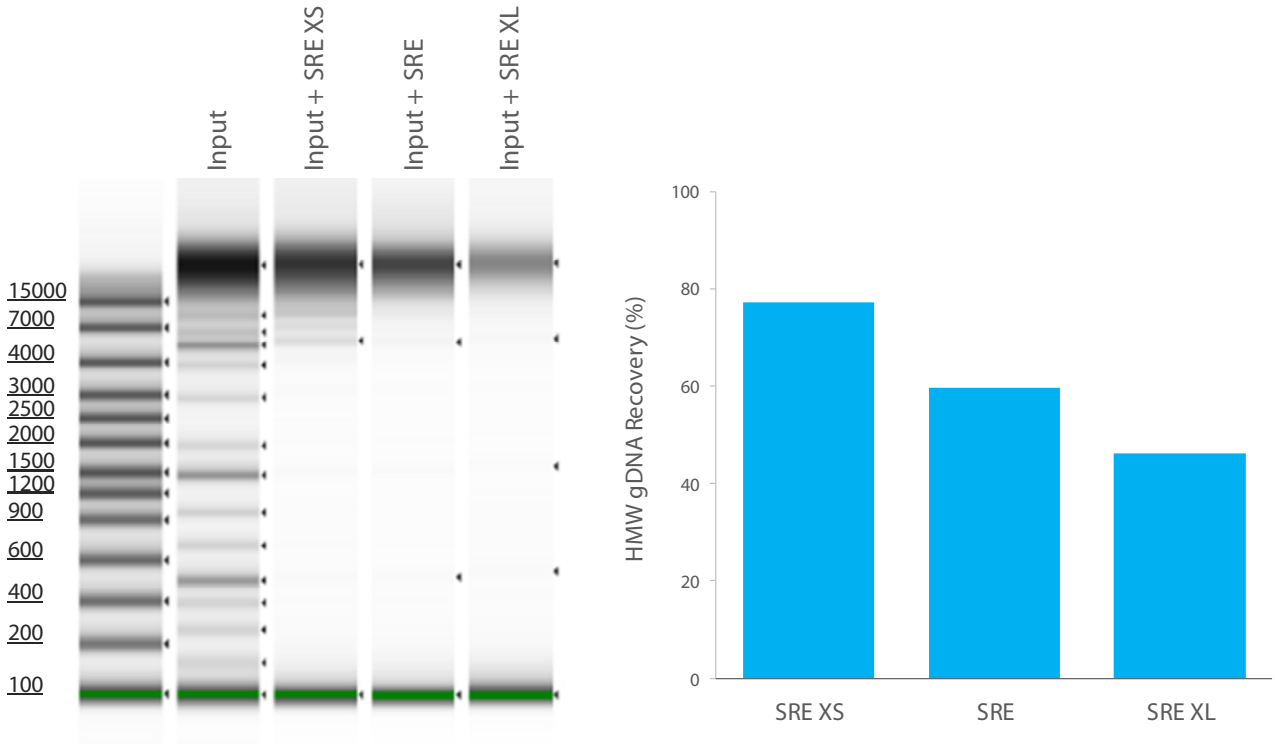


Figure 3. The sample from **Figure 1** was analyzed on Agilent Tapestation 4200. HMW gDNA recoveries were measured using the same input DNA without the ladder.

Sheared/Fragmented DNA Size Selection (SRE XS Only)

For sheared or fragmented DNA samples, only the Short Read Eliminator XS Kit should be used. Use of the Short Read Eliminator or the Short Read Eliminator XL Kits on sheared/fragmented DNA samples will likely result in very low recovery efficiency.

Figure 4 shows size selection and recovery data from DNA samples that were sheared down 10, 20, and 30 kb using Megaruptor. DNA input concentration down to 25 ng/µL can be used for all samples except the 10 kb sample, which requires 50 ng/µL to obtain suitable recovery.

The Short Read Eliminator XS can also be used on long PCR amplicons.

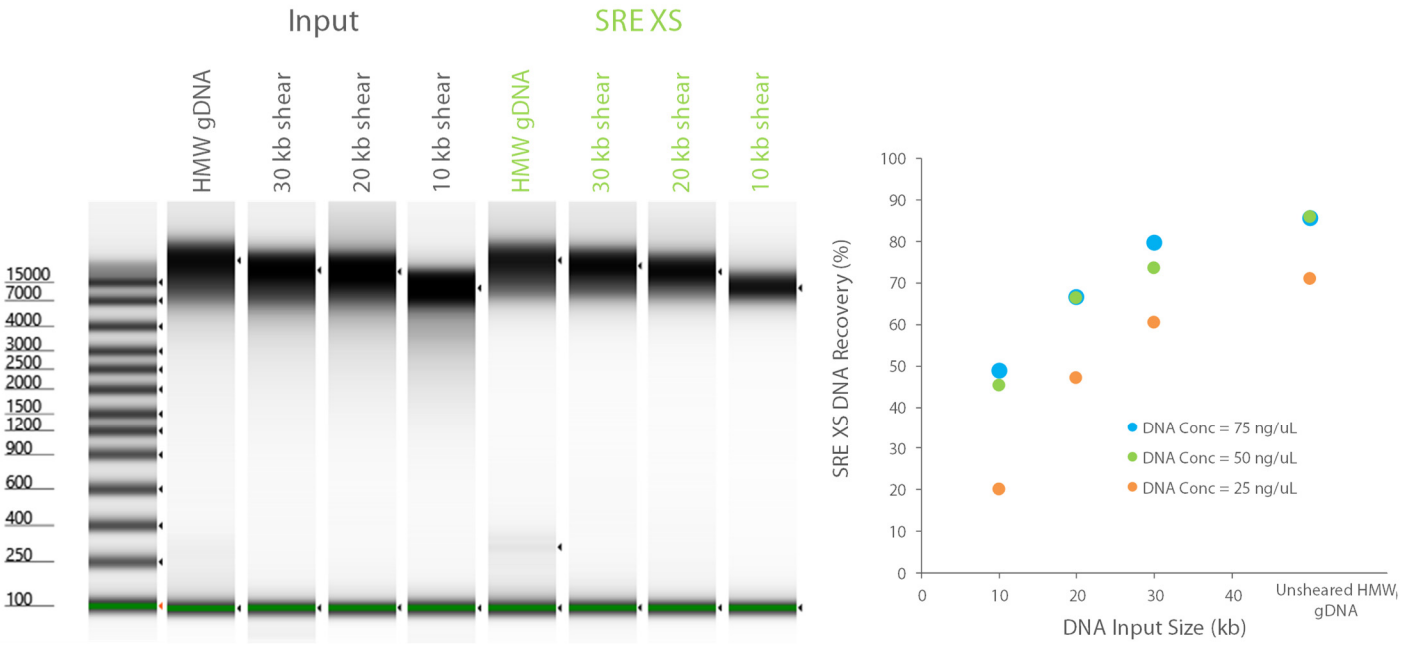
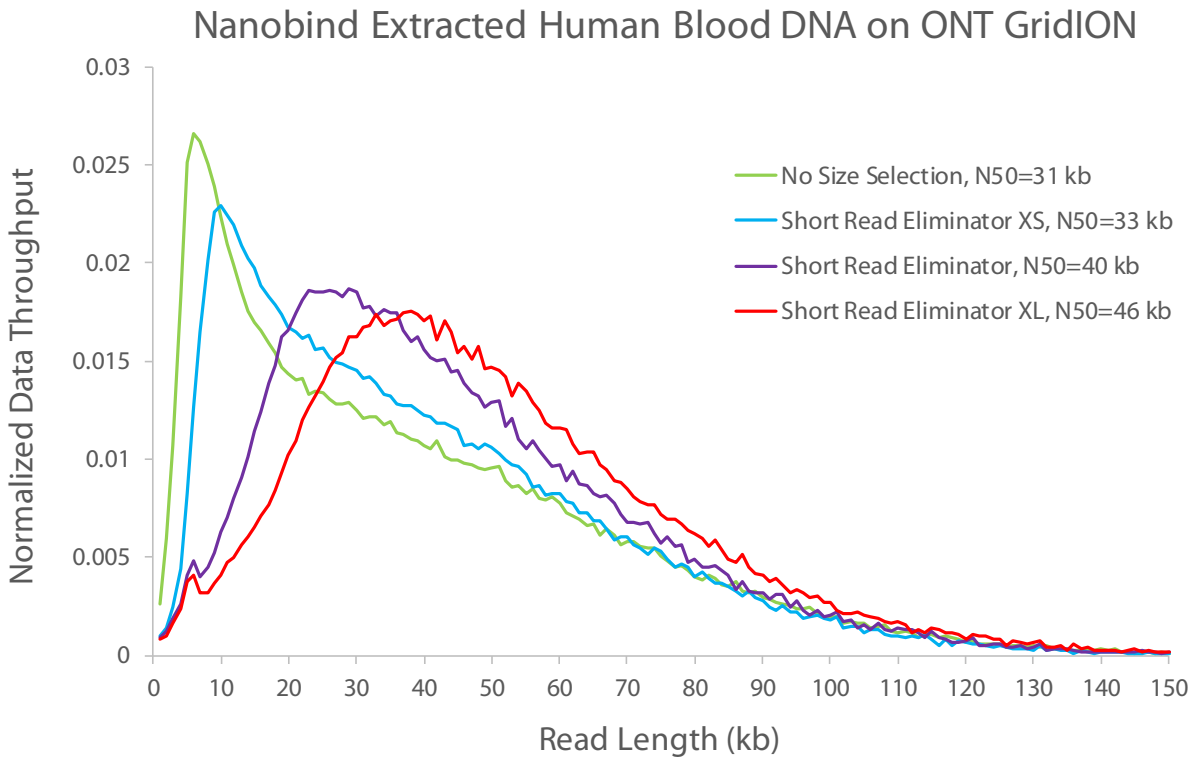


Figure 4. gDNA was extracted from GM12878 cells using the Nanobind CBB Big DNA Kit, diluted to 100 ng/µL, and sheared to 10, 20, or 30 kb using Megaruptor 2. The samples were then size selected using the Short Read Eliminator XS Kit and analyzed on Agilent Tapestation 4200. Recovery efficiency decreases with DNA length but is >50% for all samples at input concentrations ≥50 ng/µL. 25 ng/µL can be used for all samples except the 10 kb sample, unless low recovery efficiency can be tolerated.

Oxford Nanopore MinION/GridION Sequencing

HMW DNA was extracted from human blood using the Nanobind CBB Big DNA Kit and then sequenced on Oxford Nanopore GridION (FLO-MIN106D) using the Ligation Sequencing Kit (SQK-LSK109). Size selection of the HMW DNA using the Short Read Eliminator Kit just before library preparation increased N50 from 30.9 kb up to 45.5 kb depending on which version was used.

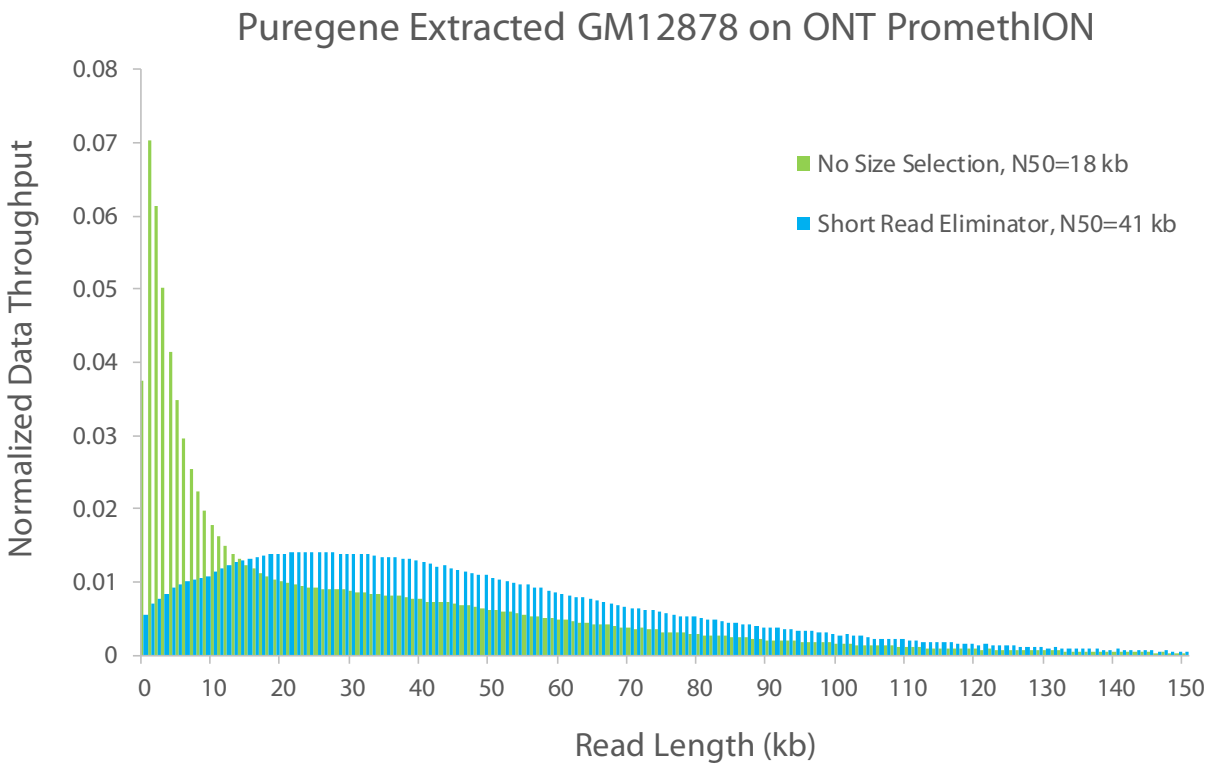


Oxford Nanopore MinION/GridION				
Extraction Method	**Size Selection**	**Shear**	**Read Length N50 (bp)**	**Throughput (Gb)**
Nanobind CBB Big DNA Kit	None	None	30,845	9.7
Nanobind CBB Big DNA Kit	Shor Read Eliminator XS	None	32,767	7.4
Nanobind CBB Big DNA Kit	Short Read Eliminator	None	39,456	7.2
Nanobind CBB Big DNA Kit	Short Read Eliminator XL	None	45,484	7.7

Figure 5. gDNA was extracted from human blood using the Nanobind CBB Big DNA Kit, size selected using each of the Short Read Eliminator Kits, and sequenced on Oxford Nanopore GridION.

Oxford Nanopore PromethION Sequencing

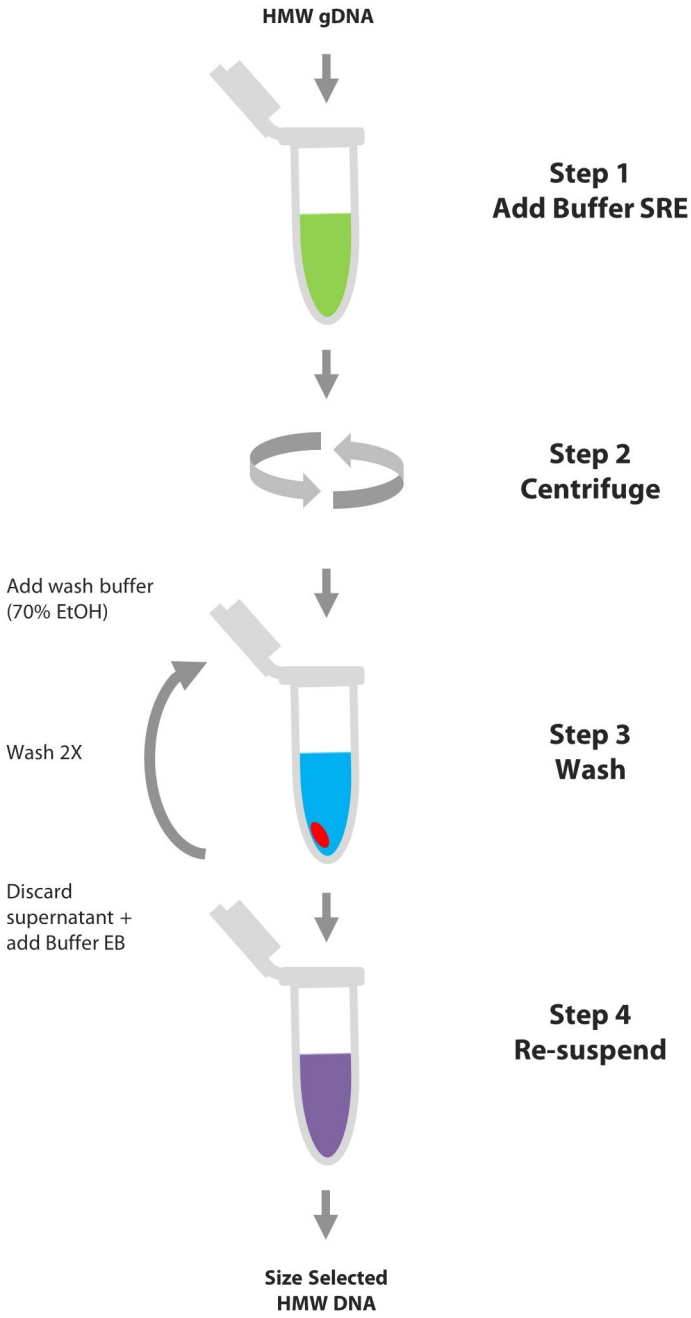
HMW DNA was extracted from GM12878 cells using Qiagen Puregene and then sequenced on Oxford Nanopore PromethION (FLO-PRO002) using the Ligation Sequencing Kit (SQK-LSK109). Size selection of the HMW DNA using the Short Read Eliminator Kit increased N50 from 17.6 kb to 40.6 kb.



Oxford Nanopore PromethION*				
Extraction Method	**Size Selection**	**Shear**	**Read Length N50 (bp)**	**Throughput (Gb)**
Qiagen Puregene	None	None	17,615	65.2
Qiagen Puregene	Short Read Eliminator	None	40,589	61.2

Figure 6. gDNA was extracted from human blood using the Qiagen Puregene, size selected using the Short Read Eliminator Kit, and sequenced on Oxford Nanopore PromethION. *Run by Oxford Nanopore.

Workflow


HMW gDNA
Step 1
Add Buffer SRE
Step 2
Centrifuge
Add wash buffer
(70% EtOH)
Wash 2X
Step 3
Wash
Discard
supernatant +
add Buffer EB
Step 4
Re-suspend
Size Selected
HMW DNA

Processing Tips

Pipetting

Load tube into centrifuge with the hinge facing toward the outside of the rotor. This will help to avoid disturbing the pellet if it cannot be seen. After centrifugation, the DNA pellet will have formed on the bottom side of the microcentrifuge tube under the hinge region. Pipette on opposite side towards the thumb lip to avoid disturbing the pellet as shown in **Figure 7**.

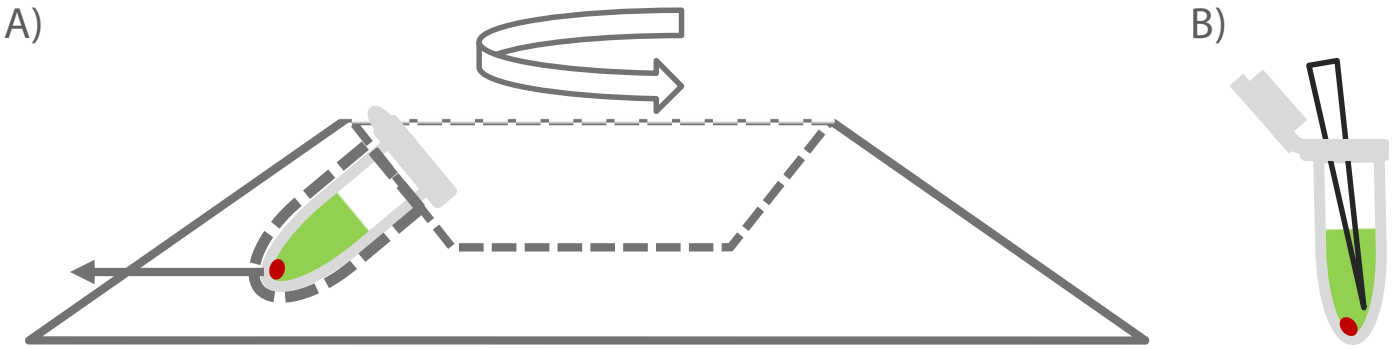


Figure 7. A) Note orientation of tube in centrifuge. Pellet will form on side of the tube facing outwards, in this case underneath the hinge region. **B)** Pipette from opposite side of tube on the thumb lip side to avoid disturbing pellet. Pellet may not be visible.

Heterogeneity and Viscosity

Recovery efficiency and size selection performance of the Short Read Eliminator Kits depends on the input DNA being homogeneous and fully in solution. HMW DNA can sometimes be difficult to re-solubilize after extraction and results in a jelly-like, inhomogeneous sample. Such samples will result in low yields and carry-over of short DNA if used with the Short Read Eliminator Kits. If the HMW DNA sample is inhomogeneous or contains viscous jellies, we recommend needle shearing with 5-10X with a 26G needle and then allowing the DNA to rest at RT overnight before beginning size selection. Sample homogeneity can be evaluated by performing triplicate concentration measurements and verifying that the concentration CV is <20%.

Size Selection Protocol for SRE and SRE XL

The following protocol details size selection of HMW gDNA prior to long read sequencing library preparation for Oxford Nanopore MinION/GridION/PromethION. The input HMW DNA should have length >48 kb and Qubit DNA concentration >50 ng/µL. **Always use wide bore pipettes**.

HMW gDNA

1. **Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration of between 50 – 150 ng/µL. Pipette sample into a 1.5 mL Eppendorf DNA LoBind tube.**
    - This concentration MUST be measured using Qubit dsDNA Broad Range Assay or equivalent.
    - Dilute sample using TE buffer (pH 8), Buffer EB, or water.
2. **Add 60 µL of Buffer SRE or Buffer SRE XL to the sample. Mix thoroughly by gently tapping the tube or by gently pipetting up and down.**
3. **Load tube into centrifuge with the hinge facing toward the outside of the rotor.**
4. **Centrifuge at 10,000 x g for 30 mins at room temperature (RT).**
    - If using a centrifuge with temperature control (*i.e.* cooling function), turn this function off by setting the temperature to 29 °C.
5. **Carefully remove supernatant from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 3).**
    - The DNA pellet will have formed on the bottom of the tube under the hinge region.
6. **Add 200 µL of the 70% EtOH wash solution to tube and centrifuge at 10,000 x g for 2 mins at RT.**
    - Do not tap or mix after adding 70% EtOH. Place tube directly into centrifuge.
7. **Carefully remove wash solution from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 3).**
8. **Repeat step 6 and step 7.**
9. **Add 50 - 100 µL of Buffer EB to the tube and incubate at room temperature for 20 minutes. Buffer volume may be adjusted to achieve desired concentration.**
10. **After incubation, gently tap the tube to ensure that the DNA is properly re-suspended and mixed.**
11. **Analyze the recovery and purity of the DNA by NanoDrop and Qubit.**

Quick Tip

Using concentrations derived from UV-Vis measurements without accounting for RNA concentrations will adversely affect yields.

Quick Tip

The DNA pellet may not be visible. Placing the tube and pipetting in the directed orientations will prevent accidentally aspirating the DNA pellet.

Quick Tip

Longer DNA can take more time to re-suspend. Heating to 50 °C or eluting for more time can help increase recoveries.

Size Selection Protocol for SRE XS

The following protocol details size selection of HMW gDNA or sheared/fragmented DNA prior to long read sequencing library preparation for Oxford Nanopore MinION/GridION/PromethION. The Qubit DNA concentration should >25 ng/µL. **Always use wide bore pipettes**.

HMW gDNA or sheared/fragmented DNA

1. **Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration of between 25 – 150 ng/µL. Pipette sample into a 1.5 mL Eppendorf DNA LoBind tube.**
    - This concentration MUST be measured using Qubit dsDNA Broad Range Assay or equivalent.
    - Dilute sample using TE buffer (pH 8), Buffer EB, or water.
2. **Add 60 µL of Buffer SRE XS to the sample. Mix thoroughly by gently tapping the tube or by gently pipetting up and down.**
3. **Load tube into centrifuge with the hinge facing toward the outside of the rotor.**
4. **Centrifuge at 10,000 x g for 30 mins at room temperature (RT).**
    - If using a centrifuge with temperature control (*i.e.* cooling function), turn this function off by setting the temperature to 29 °C.
5. **Carefully remove supernatant from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 3).**
    - The DNA pellet will have formed on the bottom of the tube under the hinge region.
6. **Add 200 µL of the 70% EtOH wash solution to tube and centrifuge at 10,000 x g for 2 mins at RT.**
    - Do not tap or mix after adding 70% EtOH. Place tube directly into centrifuge.
7. **Carefully remove wash solution from tube without disturbing the DNA pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 3).**
8. **Repeat step 6 and step 7.**
9. **Add 50 - 100 µL of Buffer EB to the tube and incubate at room temperature for 20 minutes. Buffer volume may be adjusted to achieve desired concentration.**
10. **After incubation, gently tap the tube to ensure that the DNA is properly re-suspended and mixed.**
11. **Analyze the recovery and purity of the DNA by NanoDrop and Qubit.**

Quick Tip

Using concentrations derived from UV-Vis measurements without accounting for RNA concentrations will adversely affect yields.

Quick Tip

The DNA pellet may not be visible. Placing the tube and pipetting in the directed orientations will prevent accidentally aspirating the DNA pellet.

Quick Tip

Longer DNA can take more time to re-suspend. Heating to 50 °C or eluting for more time can help increase recoveries.

Troubleshooting Guide

Recovery is poor:

- **Highly fragmented gDNA.** Recovery will be low if DNA is not HMW. Verify that a significant fraction of the input DNA is >48 kb by using PFGE or capillary electrophoresis.
- **Low input DNA concentration.** Recovery will be low if dsDNA concentration is <50 ng/µL. Verify the input gDNA concentration using Qubit dsDNA Broad Range Assay or equivalent. Using concentrations derived solely from UV-Vis measurements will often result in low recovery as the estimated DNA concentration will not account for RNA that is also present in the solution. Try increasing concentration of input DNA up to the maximum of 150 ng/µL.
- **Incorrect centrifugation speed.**
- **Incorrect centrifugation temperature.** Recovery will be impacted if centrifugation is performed at low temperature (*e.g.* 4 °C). Verify that the centrifuge is not cooling by turning off cooling or setting the temperature above ambient (*e.g.* 29 °C).
- **Heterogeneous input sample.** If input sample is heterogeneous and contains fractions of DNA that are not fully solubilized, recovery will be affected. Verify homogeneity by pipetting to ensure that no viscous jellies exist in the sample. Homogeneity can also be determined by performing triplicate concentration measurements and verifying that the CV <20%. If the sample fails these tests, needle shear the input DNA 10X using a 26G needle or pipette mix 10X using a standard P200 pipette and allow to rest overnight at RT before proceeding.
- **Non-standard DNA buffer.** This method has only been tested using DNA in solubilized in TE buffer (pH 8), Buffer EB, or water. If the DNA sample contains high levels of contaminants or salts, recovery may be affected.
- **Handling error.** The DNA pellet is often invisible. If the pellet is disturbed during the wash steps, it is possible to accidentally aspirate it into the pipette tip. Ensure that proper care is taken with tube orientation during centrifuge and pipetting steps such that pipetting is always performed on the opposite side of the tube from the pellet.

Cutoff seems too low/too high:

- **Non-standard DNA buffer.** This method has only been tested using DNA in water, TE buffer or Buffer EB. If the DNA sample contains high levels of contaminants or salts or compounds that affect DNA solubility/precipitation, size selection performance may be affected.