Feb 14, 2026

Modified size selection protocol for SRE XS (PacBio)

DOI
https://dx.doi.org/10.17504/protocols.io.3byl48exovo5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.3byl48exovo5/v1

Protocol Citation: Simona Buonanno, Ahana Maitra, Grazia Visci, Carmela Gissi 2026. Modified size selection protocol for SRE XS (PacBio). **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl48exovo5/v1

Manuscript citation:

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

Created: December 15, 2025

Last Modified: February 14, 2026

Protocol Integer ID: 235317

Keywords: HMW-DNA, Biodiversity Genomics Europe, BGE, short read eliminator, sre, sre xs, size selection, hifi pacbio sequencing, size selection protocol for sre x, pacbio protocol version, sfe001 oxford nanopore v4, modified size selection protocol, fragmented dna, oxford nanopore, size selection protocol, sre xs kit, pacbio, genomic dna, gdna, sre x, protocol of sfe ont exp, dna

Funders Acknowledgements:
Biodiversity Genomics Europe receives funding from the European Union's Horizon Europe Research and Innovation Action
Grant ID: 101059492
The Genomic Core Facility of Elixir-IT, the Italian node of the European Research Infrastructure for Life Science (UNIBA&CNR), empowering project ELIXIRNextGenIT
Grant ID: IR0000010

Disclaimer

The duration times presented are estimations only.

Abstract

This protocol describes a modified size-selection procedure applied to high-molecular-weight (HMW) genomic DNA (gDNA) or fragmented DNA using the SRE XS kit. For HiFi PacBio sequencing, size selection should be performed on gDNA prior to shearing.

This is a merged protocol based on Size selection protocol for SRE XS: first part (steps 1-4) is from PacBio protocol Version: 102-582-400 REV 05 AUG2024; second part (steps 5-14) is from protocol of SFE ONT EXP-SFE001 Oxford Nanopore v4, Last Update Aug 2025.

Acronyms:
SRE = Short read eliminator
HMW = High molecular weight
gDNA = Genomic DNA
EtOH = Ethanol

Materials

- PacBio® SRE XS kit (102-208-200)
- Agilent FemtoPulse system + approriate kit
- Qubit fluorometer + 1X HS Qubit dsDNA Assay
- Ethanol (96–100%)
- Nuclease-free water
- Pipettes + standard and wide-bore pipette tips
- Centrifuge
- 1.5 ml Eppendorf DNA LoBind tubes
- TE buffer or low TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0-8.5)
- Thermomixer

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol.

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**.

Before start

- The Qubit concentration of the input DNA should be between 25-150 ng/µL
- The DNA sample should be in TE buffer pH 8, the supplied 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 (from SRE-XS troubleshooting)

- Evaluate the fragment profile of gDNA using the Agilent FemtoPulse system
- Prepare 80% EtOH wash buffer by diluting ethanol (96–100%) with nuclease-free water.
- All buffers must be stored at room temperature (15–30°C).
- The homogeneity is essential for effective size selection, especially for HMW gDNA, so check homogeneity by taking Qubit measurements from the bottom, middle, and top of the sample (1X HS Qubit dsDNA Assay). If readings are inconsistent (e.g. >20% CV – Coefficient of variation= SD/mean), incubate the sample at 37°C for 30 minutes with gentle agitation and pipette up and down slowly using a wide-bore pipette tips. Ensure the entire sample volume is mixed, as pellets can settle at the bottom of the tube.
- Before taking the input volume, mix by pipetting up and down the DNA sample 5-6 times with a P200 pipette.
- For downstream Nanopore library preparation, we used at least 2 µg DNA as input of the size selection procedure.
- NB! The overnight incubation for elution is not included in the estimated duration for the protocol.

Size selection protocol for SRE XS (PacBio)

4h 56m

1 Use an input DNA sample with a concentration between 25-150 ng/µL and a final volume in the
range of 60-200 µL.

Pipette the sample into a 1.5 mL Eppendorf DNA LoBind tube.

2 Add an equal volume of Buffer SRE XS to the sample and mix gently by pipetting up and down 5-10 times with a P200 pipette. Name this tube R1. 2m

Note

Pipette mix the SRE XS buffer 5-10 times with P1000, before using it. Buffer SRE XS is highly viscous and difficult to pipette, so adjust the DNA sample to a volume such that the equivalent volume of buffer can be easily pipetted. If necessary, adjust the DNA volume using TE buffer pH 8, Buffer Low TE, or water.

3 Load the tube into the centrifuge with the hinge facing toward the outside of the rotor.

4 Centrifuge 10,000 x g for 00:30:00 at room temperature. 32m

Note

Ensure that centrifugation is performed at room temperature.

Using a P200 pipette, carefully remove the supernatant from tube without disturbing the DNA pellet. Place the pipette tip on the opposite side of the tube where the pellet is expected to be located (the DNA pellet will have formed on the bottom of the tube under the hinge region).

To maximise gDNA yield, **save the supernatant** in a new 1.5 mL Eppendorf DNA LoBind tube named R2.

Note

The pellet may not be visible, so it is important not to remove the full volume of the supernatant and leave ~5-10 µl to ensure the gDNA pellet is not disturbed.

5 Leave the tube R1 at RT and proceed with the next step with only the tube R2. 32m

Repeat the centrifugation and DNA pelleting steps for the retained supernatant in tube R2: follow sub-steps 5.1 - 5.4.

5.1 Place tube R2 in the centrifuge with the tube hinge facing outward.

5.2 Centrifuge 10,000 x g for 00:30:00 at room temperature.

5.3 Using a P200 standard tip, slowly and carefully remove most of the supernatant (leaving ~5–10 µl behind) by aspirating from the opposite side of the tube from the location of the pellet.

5.4 Save the third supernatant in a new 1.5 mL Eppendorf DNA LoBind tube named R3. You can leave it at 4° C until the next day, when a Qubit concentration check will be performed on R1 and R2. In case the DNA amounts in the R1 and R2 tubes were too low, repeat the centrifugation and DNA pelleting described in steps 5.1-5.4 on R3 on the next day, without any observed effect on the quality.

6 Perform an ethanol wash to the R1 and R2 sample tubes containing your DNA pellets. 32m

To each tube, follow sub-steps 6.1 - 6.3.

6.1 Slowly add 200 µl of freshly prepared 80% ethanol to the tube, without disturbing the pellet (we
recommend dispensing on the opposite side of the tube from your pellet).

6.2 Centrifuge the tubes at 10,000 x g for 00:03:00 at room temperature, ensuring the same tube orientation used in the previous centrifuge step (i.e., hinge facing outwards). 3m

6.3 Using a P200 standard tip, slowly and gently aspirate the supernatant from the opposite side of the tubes, taking care not to disturb the pellet.

Note

After the first ethanol wash, the pellet may become more visible as a white pellet at the bottom of the hinge-side of the tube:

a) If the pellet is visible, then carefully discard as much of the ethanol as possible.
b) If the pellet is not visible, leave behind ~20 µl volume of supernatant to avoid inadvertently aspirating or disturbing the pellet.

7 Repeat steps 6.1-6.3, for a total of two ethanol washes for both tubes R1 and R2. If needed, place the tubes with the pellet at 37°C for up to 1 minute to let the ethanol evaporate. 32m

Note

After the second ethanol wash, the pellet in the tube should be clearly visible in most cases and the residual ethanol can be carefully discarded using a 10 µl pipette, taking care not to disturb the pellet.

8 Elute the gDNA from each sample tube (R1 and R2), adding 50 µl of TE buffer or low TE buffer to each tube, and pipette mix using a wide-bore tip. 2m

9 Incubate the tubes in a thermomixer set to 300 rpm at 37°C for 00:30:00 . 30m

10 Leave the samples overnight at RT.

11 Gently mix the tube's contents by pipetting up and down using a P200 standard tip. Keep tubes R1 and R2 separate. 1m

12 Quantify using the Qubit 1X HS dsDNA Assay kit. 10m

Required
R1 Concentration [ng/µl]

R1 Yield [ng]

Required
R2 Concentration [ng/µl]

R2 Yield [ng]

13 Ensure the yield is consistent with the input amount and the expected yield according to the smear analysis on Agilent FemtoPulse. 2h

Note

Also other suitable methods can be used to determine the size distribution.

14 If the yield is not consistent, perform the following:

- Quantify with Qubit the third supernatant R3 and, if needed, perform on it the centrifugation and pelleting steps
- Additional mixing of R1 and R2 by pipetting and homogenization in a thermomixer at 37°C and 300 rpm for 00:30:00 .

Protocol references

* PacBio protocol Version: 102-582-400 REV 05 AUG202
* SFE ONT EXP-SFE001 Oxford Nanopore v4, Last Update Aug 2025