Removing short DNA fragments with the Short Read Eliminator (SRE) kit

Procedure & checklist

Overview

This procedure describes the workflow for DNA size selection to remove molecules <10 kb using the SRE kit on high molecular weight (HMW) DNA before shearing and library preparation.

The SRE kit is used for **rapid size selection of unsheared HMW DNA** samples. This method can significantly enhance mean read length by progressively depleting short DNA up to 10 kb. The SRE kit uses a centrifugation procedure like standard ethanol precipitation techniques. DNA is also purified during the process which could help remove contaminants for difficult samples.

Overview	
Samples	1–24
Workflow time	2 hours for up to 24 samples, 10 mins hands-on time
DNA input	
Quantity	0.6–9 µg at 10–150 ng/µL in 60 µL* of Buffer LTE, TE buffer (pH 8), or water
DNA size distribution	50% ≥30 kb

*Other volumes are possible, but the number of reactions available per kit will change as the SRE buffer should equal the sample volume.

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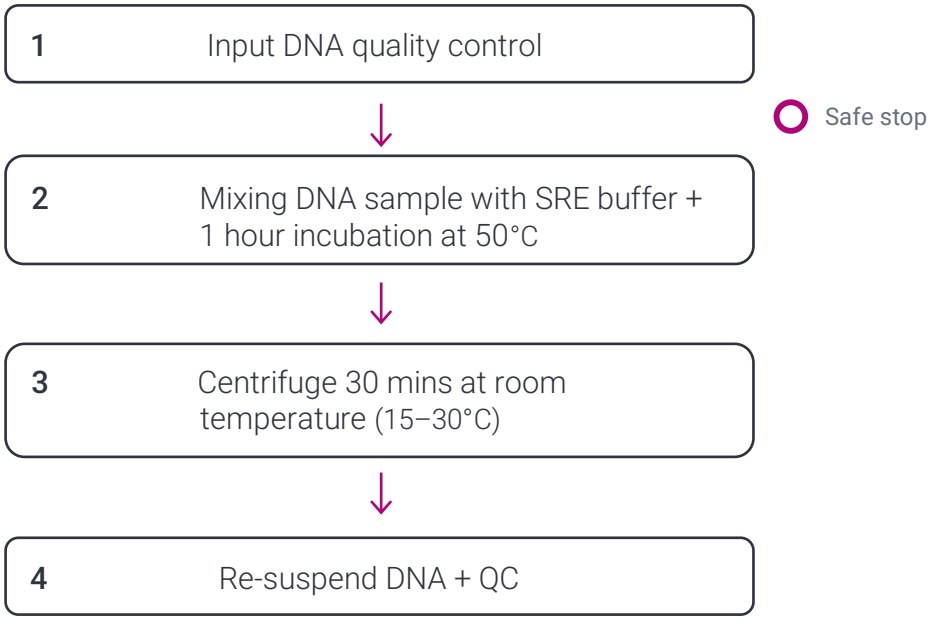
Workflow



Required materials and equipment

DNA sizing	
Femto Pulse System	Agilent Technologies M5330AA
Femto Pulse gDNA 165 kb Analysis Kit	Agilent Technologies 1002-0275
DNA quantification	
Qubit Fluorometer	Thermo Fisher Scientific Q33238
Qubit 1X dsDNA BR Assay Kit	Thermo Fisher Scientific Q33265
SRE size selection	
SRE kit	PacBio® 102-208-300
Microcentrifuge	Eppendorf 5404000413
ThermoMixer	Eppendorf 5382000023
1.5 mL DNA LoBind microcentrifuge tubes	Eppendorf 022431021

General best practices

DNA input

At least 50% of DNA should be ≥30 kb, as measured on the Femto Pulse system. This corresponds to a genome quality number (GQN) of 5.0 or higher with a 30 kb cutoff.

Start with a concentration between 10–150 ng/µL in 60 µL. This represents a mass from 0.6–9.0 µg. Sample should be in Buffer LTE, TE buffer (pH 8), or water.

Recovery should be 50% or higher.

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 1.

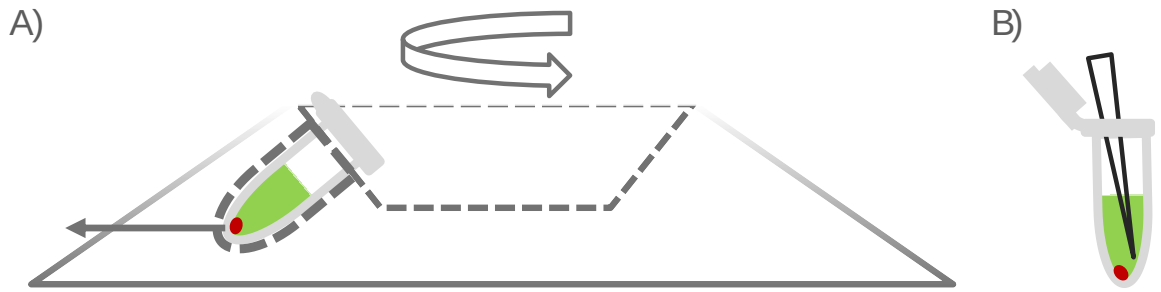


Figure 1. A) Note orientation of tube in centrifuge. Pellet will form on side of the tube facing outwards, in this case underneath the hinge region. B) Pipette from opposite side of tube on the thumb lip side to avoid disturbing pellet. Pellet may not be visible.

Heterogeneity and viscosity

Recovery efficiency and size selection performance of the SRE kit depends on the input DNA being homogeneous and fully in solution. Sample homogeneity can be evaluated by performing triplicate concentration measurements and verifying that the concentration CV is <20%.

If the HMW DNA sample is inhomogeneous, we recommend needle shearing with 5-10X with a 26G needle and then allowing the DNA to rest at room temperature overnight before beginning size selection.

Reagent and sample handling

- All buffers should be stored at room temperature

Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Procedure and checklist

1. Input DNA quality control

This protocol requires high-quality, high molecular weight (HMW) DNA. Before you begin, evaluate the quantity and size distribution of input DNA to determine whether it is suitable for the protocol.

✔	Step	Instructions
	1.1	Bring the Qubit 1X dsDNA BR working solution and standards to room temperature.
	1.2	Pulse vortex or pipette mix each sample to homogenize the DNA in solution.
	1.3	Quick-spin each sample to collect liquid.
	1.4	Measure DNA concentration with a Qubit fluorometer using the 1X dsDNA BR kit.
	1.5	Dilute each aliquot to 250 pg/µL in Femto Pulse dilution buffer based on the Qubit reading.
	1.6	Measure DNA size distribution with a Femto Pulse system using the gDNA 165kb analysis kit.
	1.7	Proceed to the next step of the protocol if sample size distribution is acceptable (GQN(30k) > 5.0).
		SAFE STOPPING POINT – Store at 4°C

2. SRE size selection

✔	Step	Instructions
	2.1	Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration between 10–150 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 LTE, or water.
	2.2	Add 60 µL of Buffer SRE to the sample. Vortex to mix for 5 seconds at max speed.
	2.3	Incubate the tube for 1 hour at 50°C in heating block.
	2.4	Load tube into centrifuge with the hinge facing toward the outside of the rotor.
	2.5	Centrifuge at 10,000 x *g* for 30 mins at room temperature. • If using a centrifuge with temperature control (i.e., cooling function), turn this function off by specifying a target temperature set point higher than ambient room temperature (e.g., 29°C or 30°C).
	2.6	Carefully remove supernatant from tube without disturbing the pellet. Place the pipette tip on the thumb lip side of the tube (see Figure 1). • The DNA pellet will have formed on the bottom of the tube under the hinge region but may not be visible. • Leaving up to 10 µL is acceptable to be sure the pellet is not disturbed.
	2.7	Add 50–100 µL of Buffer LTE to the tube and incubate at room temperature for 20 minutes.
	2.8	After incubation, pipette-mix 20 times and vortex the tube for 5s to ensure that the DNA is properly re-suspended and mixed.

2.9	Analyze the recovery and purity of the DNA by NanoDrop and Qubit. • If the recovery is lower than 50% repeat pipette-mixing 20 times and vortex for 5s.
2.10	Buffer volume may be adjusted to achieve desired concentration.
2.11	DNA can be stored in Buffer LTE at 4°C for several months. Long term storage at -20°C or -80°C can be used if necessary. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Revision history (description)	Version	Date
Initial release	01	February 2023
Updated to increase number of reactions per kit and to change buffer name from Buffer EB to Buffer LTE	02	March 2024