Introduction

The Short Fragment Eliminator (EXP-SFE001) is a new expansion pack that is recommended for users who want to size select high molecular weight (HMW) gDNA to deplete short fragments (<25 kb). The kit contains a Short Fragment Eliminator (SFE) buffer, which progressively removes fragments up to 25 kb, with fragments under 10 kb being almost completely removed.

Please note, this kit is designed to remove short DNA fragments from your sample. If there are large amounts of short fragments in the sample, this will likely result in a low yield at the end of the prep, and a low sequencing output, as they will be removed.

This kit is compatible with all of our DNA sequencing kits.

Materials

- Short Fragment Eliminator (EXP-SFE001)
- 3–10 µg of HMW DNA
- Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)
- Freshly prepared 70% ethanol in nuclease-free water
- 1.5 ml Eppendorf DNA LoBind tubes
- Qubit™ dsDNA BR Assay Kit (ThermoFisher Q32850) or equivalent QC check
- Qubit™ Assay Tubes (ThermoFisher Q32856)
- Eppendorf 5424 centrifuge (or equivalent)
- P200 pipette and wide-bore tips
- P100 pipette and wide-bore tips
- P20 pipette and wide-bore tips
- P2 pipette and tips
- Ice bucket with ice
- Heat block or water bath at 37°C
- Timer
- Qubit fluorometer (or equivalent for QC check)
- Shaking heat block (optional)

Method

Tip: We recommend using TE buffer in steps 1 and 7 rather than nuclease-free water if the library is going to be stored over a long-term period (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0).

1. In a 1.5 ml Eppendorf DNA LoBind tube, prepare 3–10 µg DNA in 60–100 µl of nuclease-free water to a final concentration of ~30–150 ng/µl.
2. Thoroughly pipette mix the SFE buffer.
3. Add an equal volume of the SFE buffer to the DNA sample and mix thoroughly by gently flicking the tube until homogenous.
4. Place the tube in the centrifuge and note the orientation of the tube within the rotor. Centrifuge the sample at 10,000 x g at room temperature for 30 minutes. Aspirate and discard the supernatant, taking care not to disturb the pellet.
   Tip: The pellet may not be visible, but it will be located on the side of the tube that was facing outwards during centrifugation. If you are concerned about aspirating the pellet, do not remove the full volume of the supernatant and leave ~10–15 µl as this will be washed out in subsequent steps.
5. Without disturbing the pellet, add 200 µl of freshly prepared 70% ethanol to the tube. Centrifuge the sample at 10,000 x g for 3 minutes, then pipette off the ethanol and discard.
   Tip: If you are concerned about aspirating the pellet, do not remove the full volume of ethanol. We recommend leaving ~10–15

µl before placing the sample in a heat block at 37°C to quickly evaporate the remaining ethanol without disturbing the pellet.

6. Repeat the previous step.
7. Add 50 µl of water to the DNA pellet and mix by gently flicking the tube.
8. Incubate the tube in a heat block at 37°C for 30 minutes. Gently agitate the solution every 5 minutes to aid with resuspension. Alternatively, use an incubated shaking heat block at 37°C, 300 rpm for 30 minutes.
9. Gently mix the tube contents by pipetting up and down using a wide-bore tip.
10. Quantify the sample three times using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to library preparation. If the Qubit measurements are not consistent, this could indicate that the DNA has not been homogeneously resuspended. If this occurs, we recommend increasing the elution time and incubating the DNA at 50°C to aid with resuspension of the DNA pellet.
11. The sample can be stored at 4°C or taken forward into library preparation.

Change log

Version	Change
v1, May 2022	Initial publication