FS Module

RK20276

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Version：N19B17v1.0

Contents

1. Components

Table 1. Components supplied with this product

	Components	24 RXN	96 RXN
	FS Pro Buffer I	120 μL	480 μL
	FS Pro Enzymes II	312 μL	1248 μL
	1X TE Buffer	1 mL	10 mL

2. Notes

2.1 Input DNA

2.1.1. Input DNA should be quantified using Qubit® or other fluorometric quantification kits.

2.1.2. Impurities in the DNA samples, such as trace amounts of residual RNAs, nucleotides, single-stranded DNAs, and other contaminants, may have an impact on fragment size. If possible, please use 2.2X magnetic beads to purify DNA samples and use 1X TE Buffer to elute purified DNA samples (Important!).

2.2 Fragmentation

2.2.1. The samples were dissolved with common solvents such as Nuclease-Free Water, 1X TE Buffer, EB (Elution Buffer), and Low-EDTA TE Buffer, which had little effect on fragmentation and could be performed normally. If the sample is dissolved using a special solvent, it is recommended to perform a pre-experiment first.

If fragmentation is not possible to the expected size, perform a 2.2X magnetic bead purification and dissolve the sample in Nuclease-Free Water.

2.2.2. For FFPE DNA samples, recommended fragmentation time is 5-10 minutes.

2.2.3. The fragmentation reaction system is sensitive to temperature; the experiment should be performed on ice. Immediately transfer the prepared reaction to the thermocycler after set-up. Return all components to -15°C to -25°C for storage as soon as possible after use.

3. Protocol

3.1 Fragmentation

3.1.1. Preheat PCR instrument to 32°C.

3.1.2. Prepare the following solution in a sterile PCR tube on ice (add FS Pro Enzymes II to the reaction system last):

Table 2. Fragmentation

	Component	Volume
	Input DNA	X μL
●	FS Pro Buffer I	5 μL
●	FS Pro Enzymes II	13 μL
○	1X TE Buffer	Up to 50 μL
	Total volume	50 μL

3.1.3. Pipe up and down or vortex to mix, and centrifuge to collect the reaction solution to the bottom of the tube.

3.1.4. Place the PCR tube on a PCR instrument preheated to 32 °C in advance.

3.1.5. If thermal inactivation is required at 32 °C after the interruption, please run according to "**Table 3. Reaction Procedure I** ". After the reaction is completed, the "end repair and A-tailing" reaction can be performed directly. For details, refer to the ABclonal Mechanical Shearing Library Prep Kit (CAT.NO.RK20208 / RK20255 / RK20256 / RK20271). If long-term storage is required, magnetic bead purification is necessary. Please refer to the procedure in "**Magnetic Bead Purification after Thermal Inactivation**".

3.1.6. If EDTA termination is required after the interruption at 32°C, please run according to "**Table 4 Reaction Procedure II**". After the reaction is completed, add 5 μL of 500 mM EDTA to each reaction system, mix thoroughly by rapid vortexing, and briefly centrifuge. The reaction products must first undergo magnetic bead purification before proceeding to the next step of "end repair and A-tailing." For details, refer to the procedure in "**Magnetic Bead Purification after EDTA Termination**".

Table 3. Reaction Procedure I

Temperature	Time
32℃	5-25 min
72℃	15 min
4℃	Hold (< 1h)

Table 4 Reaction Procedure II

Temperature	Time
32℃	5-25 min
4℃	Remove immediately.

3.2 Purification

Magnetic Bead Purification after Thermal Inactivation

3.2.1. After the completion of step 3.1.5, add 100 μL (2X) AFTMag NGS DNA Clean Beads to each reaction system, mix thoroughly, and incubate at room temperature for 5 minutes.

3.2.2. Place the PCR tube on a magnetic stand and let it sit for 2 minutes. Once the solution becomes clear, remove the supernatant (be careful not to disturb the magnetic beads).

3.2.3. Add 200 μL of 80% ethanol to wash the magnetic beads. After incubating for 30 seconds, remove the supernatant.

3.2.4. Repeat step 3.2.3.

3.2.5. Keep the PCR tube on the magnetic stand and use a 10 μL pipette to remove any residual ethanol from the bottom of the tube. Open the tube cap and dry until all ethanol has evaporated.

3.2.6. Resuspend the magnetic beads in 21 μL of Nuclease-Free Water, and let the tube sit at room temperature for 1 minute to allow the DNA on the beads to be fully released.

3.2.7. Place the PCR tube on the magnetic stand for 2 minutes, then transfer 20 μL of the supernatant to a new PCR tube for storage, which can be used for subsequent experiments. The purified DNA fragmentation product can be stored long-term at -20°C or can be

directly used with a conventional DNA library construction kit for library preparation experiments.

Magnetic Bead Purification after EDTA Termination

3.2.1. After the completion of step 3.1.6, add 110 μL (2X) AFTMag NGS DNA Clean Beads to each reaction system, mix thoroughly, and incubate at room temperature for 5 minutes.

3.2.2. Place the PCR tube on a magnetic stand and let it sit for 2 minutes. Once the solution becomes clear, remove the supernatant (be careful not to disturb the magnetic beads).

3.2.3. Add 200 μL of 80% ethanol to wash the magnetic beads. After incubating for 30 seconds, remove the supernatant.

3.2.4. Repeat step 3.2.3.

3.2.5. Keep the PCR tube on the magnetic stand and use a 10 μL pipette to remove any residual ethanol from the bottom of the tube. Open the tube cap and dry until all ethanol has evaporated.

3.2.6. Resuspend the magnetic beads in 21 μL of Nuclease-Free Water, and let the tube sit at room temperature for 1 minute to allow the DNA on the beads to be fully released.

3.2.7. Place the PCR tube on the magnetic stand for 2 minutes, then transfer 20 μL of the supernatant to a new PCR tube for storage, which can be used for subsequent experiments. The purified DNA fragmentation product can be stored long-term at -20°C or can be directly used with a conventional DNA library construction kit for library preparation experiments.

4. Appendix

Table 5. Recommended fragmentation time for expected-insert size (32℃)

Expected-insert size	Fragmentation time	Adjust fragmentation time
225 bp	20 min	18-25 min
250 bp	15 min	13-17 min
300 bp	10 min	8-12 min
500 bp	5 min	4-6 min

Note: The above recommended time was validated using 100ng NA12878 as a template. When using other types of high-quality DNA for library construction, the distribution range of fragmentation products is not much different within the same fragmentation time. For samples with severe degradation such as FFPE, the fragmentation time can be appropriately shortened, generally about 5 min.

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