FS Pro DNA Lib Prep Kit V2

RK29832



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Contents

1. Introduction 2

2. Components 3

3. Storage 3

4. Applications 4

5. Additional Materials Required 4

6. Notes 5

7. Protocol 9

8. Appendix 15

9. Appendix Table 17

1. Introduction

The FS Pro DNA Lib Prep Kit V2 is designed for DNA library preparation for next generation sequencing (NGS) on Illumina or MGI platforms. This kit integrates DNA fragmentation with end repair and dA tailing into one step, and it can be directly followed by adapter ligation without purification. With greatly simplified experimental flow, the entire experiment can be completed within 2 hours. This kit is compatible with DNA samples from different species and different origins. Optimization of reagents and protocol greatly reduced rates of false positives, significantly improving sequencing yield and overall results, including with FFPE DNA samples. This kit is suitable for the construction of PCR-Free libraries (e.g., 100 ng of high-quality genomic DNA).

The components of the kit have undergone strict quality control, mainly including component contamination test, functional test verification, application scenario testing and product batch stability test, etc., to minimize pathogenic bacteria residue and be suitable for pathogenic microorganism detection.

2. Components

Table 1. Components supplied with this product

Module		Components	24 RXN	96 RXN
Fragmentation, End Repair & dA Tailing		FS Pro Buffer I	120 μL	480 μL
		FS Pro Enzymes II	312 μL	1248 μL
		Nuclease-Free water	2 mL	10 mL
		1X TE Buffer	2 mL	10 mL
Adapter Ligation		FS Pro Ligation Buffer II	480 μL	1920 μL
		Ligase Enzymes	120 μL	480 μL
Amplification		2X PCR Mix	600 μL	2400 μL
		10X ILM PCR Primers	120 μL	480 μL

3. Storage

Transportation and storage: the FS Pro DNA Lib Prep Kit V2 must be stored at -15°C to -25°C. Because this kit is sensitive to temperature, dry ice or dry ice combined with ice packs should be used for long-distance transportation.

4. Applications

FS Pro DNA Lib Prep Kit V2 is suitable for DNA library preparation for NGS. The product includes a fragmentation, end repair and dA-tailing module, an adapter ligation module, and a PCR amplification module. This kit is compatible with various types of DNA template (genomic DNA, FFPE DNA, etc.) and 1 ng to 1000 ng of input DNA. In summary, the product is recommended to use for the following application fields:

① Whole genome sequencing.

②Exon sequencing and targeted sequencing, including Roche® NimbleGen ™ SeqCap ™ EZ, Agilent SureSelect, Illumina TruSeq®, IDT X Gen ™ Lockdown™ Probes, or other hybridization probes.

③ Metagenomic sequencing.

5. Additional Materials Required

Purification Beads: AFTMag NGS DNA Clean Beads (ABclonal, Cat. No. RK20257).

DNA quality control: Agilent Bioanalyzer, or comparable method to assess the quality of DNA library.

DNA Adapters for Illumina platform:

Unique Dual Index for Illumina (ABclonal, Cat. No. RK21622-RK21627).

RK21622-RK21627 products provide truncated adapters with an 8 bp Unique Dual Index at two ends.

Unique Dual Index Adapter Plate for Illumina (ABclonal, Cat. No.

RK21706-RK21709).

RK21706-RK21709 products provide 8 bp single-index full adapters.

DNA Adapters for MGI platform:

Truncated DNA Adapter (UDI) Kit for MGI (ABclonal, Cat. No. RK21687-RK21689)

RK21687-RK21689 products provide truncated adapters with an 10 bp Unique Dual Index at two ends.

Truncated DNA Adapter (UDI) Plate for MGI (ABclonal, Cat. No. RK21721-RK21724).

RK21721-RK21724 products provide truncated adapters with an 10 bp Unique Dual Index at two ends

Other Materials: Nuclease-Free Water , 100% ethanol, Vortex mixer, Low adsorption EP tubes, PCR tubes, Magnetic stand, PCR instrument.

6. Notes

6.1 Input DNA

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

6.1.2 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!*).

6.2 Fragmentation

6.2.1 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.

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

6.2.3 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.

6.3 Adapters

6.3.1 This kit contains library construction components and universal PCR primers. Adapter kits need to be purchased separately.

6.3.2 The amount of the adapters used affects ligation efficiency and library yield. Please refer to **Table 2** for the recommended adapter concentrations for different DNA inputs.

Table 2. Recommended adapter concentrations for libraries prepared

Input DNA	Dilution Ratio	Working Concentration
1 μg~50 ng	Undiluted	15 μM
49 ng~25 ng	1:2	7.5 μM
24 ng~10 ng	1:5	3 μM
9 ng~5 ng	1:10	1.5 μM
< 5 ng	1:20	0.75 μM

6.4 Bead-based Purification and Optional Size Selection

6.4.1 The magnetic beads should be equilibrated to room temperature for 30 minutes prior to use to optimize sample recovery and sorting efficiency.

6.4.2 Before use, ensure the magnetic beads are fully resuspended by vortexing or pipetting up and down several times.

6.4.3 80% ethanol should be freshly prepared.

6.4.4 Inadequate drying may lead to residual ethanol which may affect subsequent experiments. Excessive drying may lead to cracking of magnetic beads and decrease in recovery efficiency.

6.4.5 It is recommended to elute DNA from beads with 1X TE Buffer for stable preservation.

6.4.6 Size selection is an optional step and, if desired, should be performed following the purification step after adapter ligation. It is not recommended to perform size selection after adapter ligation without purification because the ligation system contains a high concentration of PEG.

6.4.7 Size selection inevitably leads to a loss of sample material. If it is required, an input DNA amount greater than 50 ng is recommended.

6.5 Library Amplification

The library amplification step requires strict control of the number of amplification cycles. Insufficient number of amplification cycles leads to decreased library yield; excessive amplification leads to unwanted artifacts. **Table 3** shows recommended amplification cycles for libraries prepared to obtain 1 μg library.

Table 3. Recommended cycle numbers for 1 ng–1 μg of input DNA

Input DNA（ng）	Number of cycles required to generate 1 μg library*
1000	2-3
500	3-4
250	4-5
100	5-6
50	6-7
25	7-9
10	9-11
1	13-15

*Note: Add an additional 1-3 cycles when using FFPE DNA samples.

7. Protocol

Step1 Fragmentation, End Repair & dA Tailing

1.1 Preheat PCR instrument to 32℃.

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

	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

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

1.4 Place the PCR tube on a PCR instrument preheated to 32℃ in advance and run the following reaction program. Set the temperature of the hot lid to 82℃, and hold at 4℃ for no more than 1 hour.

Temperature	Time
32℃	5-25 min
72℃	30 min
4℃	Hold (< 1h)

Table 4. 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.

Step 2. Adapter Ligation

2.1 Equilibrate the AFTMag NGS DNA Clean Beads to room temperature (for at least 30 minutes). Suspend the beads thoroughly by vortexing.

2.2 Dilute the Adapter to appropriate concentration using Low-EDTA TE Buffer according to Table 2.

2.3 Prepare the following reaction system on ice. Mix FS Pro Ligation Buffer II and Ligase Enzymes thoroughly by gently pipetting up and down, briefly centrifuge to collect to the bottom of the tube, and place on ice for use. Working Adapter should be added separately. Mix the prepared ligation reaction system well and centrifuge briefly.

	Component	Volume
	End Prep Reaction Mix (Step 1.4)	50 μL
○	FS Pro Ligation Buffer II	20 μL
○	Ligase Enzymes	5 μL
	Working Adapter（Table 2）	5 μL
	Total volume	80 μL

2.4 Place the PCR tube on the PCR instrument and run the following reaction program. The heated lid setting is off.

Temperature	Time
22℃	15 min
4℃	∞

2.5 Pipet 64 μL (0.8X) of beads into 80 μL of the Adapter Ligation products. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

2.6 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, remove the supernatant without disturbing the beads.

2.7 Keeping the PCR tube on the magnetic stand, add 200 μL of 80% ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

2.8 Repeat **Step 2.7**.

2.9 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 μL pipette. Dry the sample until the residual ethanol has just evaporated.

2.10 Take the tube out of the magnetic stand. Add 22 μL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

2.11 Keeping the PCR tube on the magnetic stand, wait for 2 minutes. Transfer 20 μL of the supernatant to a new PCR tube.

2.12 For product needing size-selection, proceed to size-selection according to **8. Appendix 8.1 Size selection (Optional)**. For product with no need for size selection, proceed to library amplification directly.

Note: The product resulting from Adapter ligation after purification can be stable for approximately 1- 2 weeks at 4 °C/-20 °C.

Step 3. Library Amplification

3.1 Prepare the following PCR Reaction System:

Table 5. PCR Amplification Reaction Setup For Truncated Adapters

	Component	Volume
	Adapter-Ligated DNA	20 μL
●	2X PCR Mix	25 μL
○	UDI Primer（MGI UDI Primer）	5 μL
	Total volume	50 μL

Table 6. PCR Amplification Reaction Setup For Full-length Adapter

	Component	Volume
	Adapter-Ligated DNA	20 μL
	2X PCR Mix	25 μL
●	10X PCR Primers（MGI PCR Primer Mix）	5 μL
●	Total volume	50 μL

3.2 Pipet up and down to mix well and centrifuge briefly.

3.3 Place the tube on the thermocycler and run the following reaction program. Set the temperature of the heated lid to 105℃.The recommended number of library amplification cycles is shown in **Table 3**.

Temperature	Time	Cycles
98℃	1 min	1
98℃	10 s	
60℃	30 s	2-15 PCR Cycles
72℃	30 s	
72℃	1 min	1
4℃	∞	1

3.4 Pipet 50 μL (1X) of beads into 50 μL of the Library Amplification products. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

3.5 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, remove the supernatant without disturbing the beads.

3.6 Keeping the PCR tube on the magnetic stand, add 200 μL of 80%

ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

3.7 Repeat **Step 3.6**.

3.8 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 μL pipette. Dry the sample until the residual ethanol has just evaporated.

3.9 Take the tube out of the magnetic stand. Add 32 μL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

3.10 Returning the PCR tube to the magnetic stand, wait for 2 minutes. Transfer 30 μL of the supernatant to a new PCR tube.

Store the final library at -20 ℃.

Note: The purified amplification products can be stable for approximately 1- 2 weeks at 4 ℃/-20 ℃.

8. Appendix

8.1 Size selection (Optional)

Table 7. Size Selection of Library

Expected Insert Size (bp)	350~400	300~350	280~330
Expected library Size (bp)	475~525	425~475	400~450
1st volume ratio (Beads: DNA)	0.6X (60 μL)	0.65X (65 μL)	0.7X (70 μL)
2nd volume ratio (Beads: DNA)	0.15X (15 μL)	0.15X (15 μL)	0.15X (15 μL)

Size selection protocols are optimized for ratios of 0.65X/0.15X.

8.1.1 Adjust product of step 2.11 to 100 μL using Nuclease-Free Water. Pipet 65 μL (0.65X) of beads into the 100 μL of adjusted solution. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

8.1.2 Place the PCR tube on a magnetic stand and wait for 2 minutes. After the solution clarifies, transfer the supernatant to a new tube without disturbing the beads.

8.1.3 Pipet 15 μL (0.15X) of beads into the supernatant from 8.1.2. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

8.1.4 Place the PCR tube on a magnetic stand and wait for 2 minutes. After

the solution clarifies, remove the supernatant without disturbing the beads.

8.1.5 Keeping the PCR tube on the magnetic stand, add 200 μL of 80% ethanol to rinse the magnetic beads and incubate for 30 s, then remove the supernatant without disturbing the beads.

8.1.6 Repeat **Step 8.1.5**.

8.1.7 Keeping the PCR tube on the magnetic stand, remove residual ethanol with 10 μL pipette. Dry the sample until the residual ethanol has just evaporated.

8.1.8 Take the tube out of the magnetic stand. Add 22 μL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting and incubate for 1 min at room temperature.

8.1.9 Returning the PCR tube to the magnetic stand, wait for 2 minutes. Transfer 20 μL of the supernatant to a new PCR tube.

9. Appendix Table

Table 8. List of adapters for the Illumina / MGI platforms

Index	Name	Cat.No
UDI (8-base)	Unique Dual Index for Illumina MiniSet (8 indices)	RK21622
	Unique Dual Index for Illumina MidiSet (24 indices)	RK21623
	Unique Dual Index for Illumina Set_A (48 indices)	RK21624
	Unique Dual Index for Illumina Set_B (48 indices)	RK21625
	Unique Dual Index for Illumina Set_C (48 indices)	RK21626
	Unique Dual Index for Illumina Set_D (48 indices)	RK21627
UDI (8-base)-UMI	Unique Dual Index (with UMI) for Illumina MidiSet (24 indices)	RK21701
	Unique Dual Index (with UMI) for Illumina Set_A (48 indices)	RK21702
	Unique Dual Index (with UMI) for Illumina Set_B (48 indices)	RK21703
UDI (10-base)	Truncated DNA Adapter (UDI) Kit for MGI MiniSet	RK21686
	Truncated DNA Adapter (UDI) Kit for MGI MidiSet	RK21687
	Truncated DNA Adapter (UDI) Kit for MGI Set A	RK21688
	Truncated DNA Adapter (UDI) Kit for MGI Set B	RK21689
Single Index (10-base)	Full DNA Adapters Kit for MGI MiniSet	RK21676
	Full DNA Adapters Kit for MGI MidiSet	RK21677
	Full DNA Adapters Kit for MGI Set A	RK21678
	Full DNA Adapters Kit for MGI Set B	RK21679

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