DNA Frag Module

RK20260

www.abclonal.com

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Contents

1. Introduction

The DNA Frag Module has been optimized to convert intact DNA to fragmented DNA by generating dsDNA breaks in a time-dependent manner to yield fragments of desired size. The fragmented DNA product is suitable for subsequent DNA library construction. This kit has broad compatibility with DNA samples from different species. For formalin-fixed paraffin-embedded (FFPE) samples, this kit demonstrates excellent performance and library yield. This module contains DNA Frag Reaction Buffer, DNA Frag Enzyme Mix with 1X TE Buffer Reaction Supplement, and 500 mM EDTA.

2. Components

All components should be stored at -20°C. The shelf life of each reagent is one year when stored properly.

Table 1. Components supplied with this product

	Components	8 RXN	24 RXN	96 RXN
●	DNA Frag Reaction Buffer	32 µL	96 µL	384 µL
●	DNA Frag Enzyme Mix	32 µL	96 µL	384 µL
○	1X TE Buffer	320 µL	960 µL	3840 µL
○	500 mM EDTA	40 µL	120 µL	480 µL

3. Storage

Transportation and storage: DNA Frag Module kits must be stored at -15°C to -25 °C. As this kit is sensitive to temperature, dry ice or dry ice combined with ice packs should be used when possible for long-distance transportation.

4. 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

Other Materials: Nuclease-Free Water; 10X TE Buffer; 100% ethanol; Vortex mixer; Low adsorption EP tubes; PCR tubes; Magnetic stand; thermocycler.

5. Notes

5.1 Input DNA

5.1.1 For successfully generating desired fragment sizes, it is recommended to use 1X TE Buffer to dissolve DNA samples (Important!).

5.1.2 Input DNA should be quantified using Qubit® or other fluorometric quantification kits for DNA samples.

5.1.3 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 1.8X magnetic beads to purify DNA samples and use 1X TE Buffer to dissolve DNA samples (Important!).

5.2 Fragmentation

5.2.1 The fragmentation system must be complemented by 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8,0). Using water will severely shorten the fragment size.

5.2.2 If the DNA samples have been dissolved in an EDTA-Free solution, please prepare the reaction system by using 10X TE Buffer according to the following table:

Table 2. Input DNA Fragmentation (EDTA-Free solution)

	Component	Volume
	Input DNA (EDTA-Free solution)	X µL
	DNA Frag Reaction Buffer	4 µL
	DNA Frag Enzyme Mix	4 µL
	10X TE Buffer	3.2 µL
	Nuclease-Free Water	Up to 40 µL
	Total volume	40 µl

5.2.3 If the final concentration of EDTA in the DNA sample is greater than 1 mM, please pretreat the DNA sample by purifying with 2.2X magnetic beads and the DNA sample elute with 1X TE Buffer.

5.2.4 It is recommended to use FFPE DNA QC Kit (ABclonal, Cat. No. RK20229) to treat FFPE DNA samples. For high quality FFPE samples

(grade 1-2), follow the protocol indicated for genomic DNA; for FFPE DNA samples (grade 3-5), please reduce the fragmentation time to 5-10 minutes.

5.2.5 Fragmentase is sensitive to temperature; the experiment should be performed on ice. Place the prepared reaction in the PCR instrument for immediately after preparation. All module components should be stored at -15°C to -25°C as soon as possible after use.

5.3 Beads

5.3.1 Equilibrate the magnetic beads to room temperature for 30 minutes before use to optimize sorting efficiency and sample recovery.

5.3.2 Before use, ensure the magnetic beads are fully resuspended by vortexing or pipetting several times.

5.3.3 80% ethanol should be freshly prepared.

5.3.4. 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.

5.4 Without Beads Purification after Fragmentation

5.4.1 If the DNA fragments are immediately used for library construction after fragmentation, run the reaction program described in Table 3 then processed immediately according to protocol included in the Rapid Plus DNA Lib Prep Kit for Illumina V2 (ABclonal, Cat. No. RK20255) or other equivalent kits.

Table 3. Thermal cycler program for Fragmentation with no need for purification

Temperature	Time
32℃	5-30 min
72℃	15 min
4℃	∞

5.4.2 Library size after the purification with magnetic beads versus the library size without purification will be slightly different. Recommendations for reaction time to achieve various target fragment sizing can be found in the following table.

Table 4. Recommended fragmentation time for expected-insert size (32℃)

Expected-insert size	Fragmentation time	Adjusted fragmentation time
150 bp	20 min	18-25 min
200 bp	15 min	13-17 min
250 bp	10 min	8-12 min
300 bp	8 min	6-10 min
400-600 bp	5 min	4-6 min

6. Protocol

Step 1. Fragmentation

1.1 Prepare the following solution in a sterile PCR tube on ice (add DNA Frag Enzyme Mix to the reaction system last):

Table 5. Fragmentation Reaction Setup (per sample)

	Component	Volume
	Input DNA (dissolved in 1X TE Buffer)	X µL
●	DNA Frag Reaction Buffer	4 µL
●	DNA Frag Enzyme Mix	4 µL
○	1X TE Buffer	Up to 40 µL
	Total volume	40 µL

Note: Input DNA must be dissolved in 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8, 0). If the DNA sample has been dissolved in an EDTA-Free solution, please prepare the reaction system by using 10X TE Buffer according to the instructions in **5. Notes, 5.2 Fragmentation, 5.22**.

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

1.3 Place the PCR tube on the thermocycler and follow the reaction program described in Table 6. Set the temperature of the heated lid to 75° C. Recommended fragmentation times to achieve expected insert sizes are shown in Table 7.

Table 6. Fragmentation Reaction Program

Temperature	Time
32℃	5-30 min
4℃	∞

Table 7. Recommended fragmentation time for expected-insert size (32℃)

Expected-insert size	Fragmentation time	Adjusted fragmentation time
150 bp	30 min	25-35 min
180 bp	20 min	18-25min
200 bp	15 min	13-17 min
270 bp	10 min	8-12min
320 bp	8 min	6-10 min
400-600 bp	5min	4-6min

1.4 Place the reaction tube on ice immediately to terminate the reaction.

Step 2. Purification with Magnetic Beads

2.1 Equilibrate the AFTMag NGS DNA Clean Beads to room temperature for at least 30 minutes.

2.2 Pipet 5 μL of 500 mM EDTA into 40 μL of Fragmentation product. Mix thoroughly by gently pipetting up and down, then briefly centrifuge to collect to the bottom of the tube.

2.3 Pipet 90 μL (2X) of AFTMag NGS DNA Clean Beads into each reaction tube. Mix thoroughly by vortexing or pipetting up and down. Incubate at room temperature for 5 minutes.

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

2.5 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.

2.6 Repeat Step 2.5.

2.7 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.

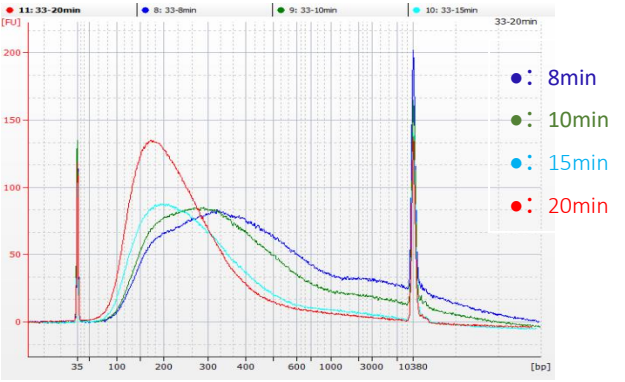
2.8 Remove the sample tube from magnetic stand. Add 21 µL of Nuclease-Free Water to resuspend the magnetic beads. Mix thoroughly by vortexing or pipetting, then incubate for 1 min at room temperature.

2.9 Place the PCR tube on the magnetic stand and wait for 2 minutes. Transfer 20 µL of the supernatant to a new PCR tube. Process Library Preparation immediately or store products at -20 ° C. The selection of library preparation kits is detailed in 7. Appendix 7.5. Options for Library Preparation.

7. Appendix

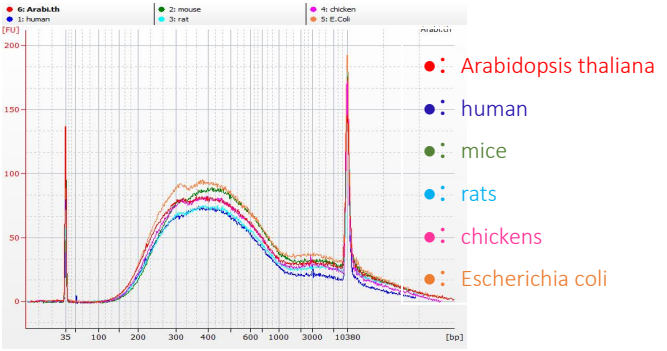
7.1 Fragmentation as determined by reaction time.

100 ng human blood gDNA was starting template for library construction using this kit. The fragmentation conditions were at 32°C for 8/10/15/20 min, and the distribution of final libraries is shown below.



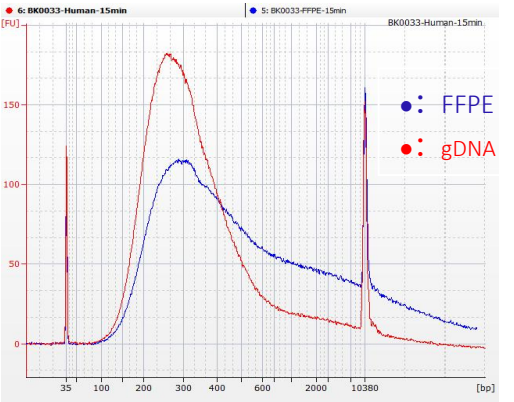
7.2 Fragmentation of DNA samples from different species

100 ng DNA from Arabidopsis thaliana, human, mice, rat, chicken and Escherichia coli were used as starting templates for library construction using this kit. The fragmentation condition for all samples was 32°C for 15 min, and the distribution of final libraries is shown below.



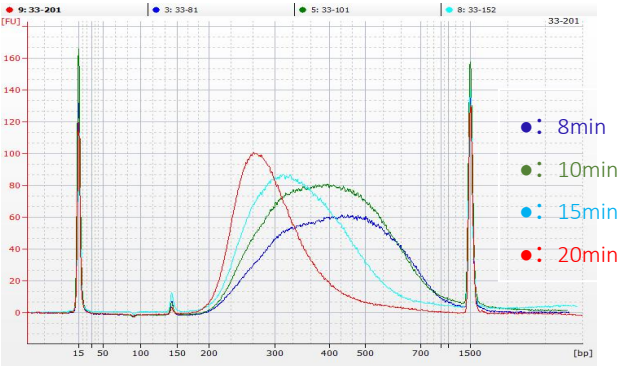
7.3 FFPE DNA / gDNA Fragmentation.

100 ng FFPE DNA and 100 ng gDNA were the starting templates for library construction using this kit. Samples were fragmented at 32°C for 15 min, and the distribution of final libraries is shown below.



7.4 Fragmentation without Purification for Library Preparation

100 ng human blood gDNA was the starting template for library construction using this kit. The fragmentation conditions were at 32°C for 8/10/15/20 min, 75°C for 15 min, followed immediately with Library Preparation immediately according to the protocol outlined in the Rapid Plus DNA Lib Prep Kit for Illumina V2 (ABclonal, Cat. No. RK20255). The distribution of final libraries is shown below.



7.5 Options for Library Preparation

Following this module, ABclonal offers several DNA Library Preparation kits for subsequent library construction after DNA fragmentation:

Table 7. ABclonal DNA Library Preparation kits for DNA Frag Module

Kit	Cat.NO
Rapid Plus DNA Lib Prep Kit for Illumina V2	RK20255
Rapid CE DNA Lib Prep Kit for Illumina V2	RK20254
Rapid Plus DNA Lib Prep Kit for MGI V2	RK20256
Scale ssDNA-seq Lib Prep Kit for Illumina V2	RK20228

Note: The library preparation kits should be used with corresponding adapter kits. Please refer to the corresponding instructions for specific operating procedures. For additional details regarding these kits, please consult us for technical support.

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