5X WGS Fragmentation Mix

Instructions for Use

Product Number

Y9410L

Product Description

The 5X WGS Fragmentation Mix is an enzyme mix to perform DNA fragmentation, end-repair and dA-tailing in one step, thereby greatly simplifying the workflow, reducing the total reaction time and hands-on time.

Kit Contents

Part Number	Y9410L (24 Reactions)
5X WGS Fragmentation Mix	240 µL
10X Fragmentation Buffer	250 µL
Enhancer Buffer	250 µL

5X WGS Fragmentation Mix (Y9410): Contains enzyme mix to perform DNA fragmentation, End-Repair and dA-tailing in one step.

Supplied with:
10X Fragmentation Buffer (B0330): Reaction buffer
Enhancer Buffer (B0340) - Enhances performance on low input DNA or DNA with 1 mM EDTA

Reagents Not Supplied

WGS Ligase *L6030-W-L can be purchased from Enzymatics*
2X HiFi PCR Master Mix (P7670)
Nuclease free water

Storage and Handling

All reagents should be stored between -25°C and -15°C

General Precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microfuge tubes and pipette tips that are certified sterile, DNase- and RNase-free.

- Before starting, wipe down work area and pipettes with an RNase and DNA cleaning product such as RNase Away® (Molecular BioProducts, Inc. San Diego, CA).
- For consistent library amplification, ensure the thermal cycler used in this protocol is in good working order and has been calibrated to within the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at -20°C and plan your workflow accordingly.
- Enzyme-based DNA fragmentation is sensitive to many factors, such as reaction temperature, time, and setup conditions, as well as the input DNA. We strongly recommend users practicing the protocol and optimizing the parameter (reaction time) using the same or similar DNA samples.

Before you Begin

Thaw reagents on ice. Once reagents are thawed, mix the 5X Fragmentation Enzyme Mix by finger flicking (**do not vortex to mix**), and mix other components by quick vortexing to avoid any localized concentrations.

Input Nucleic Acid Concentration and Purification

- **Recommended Input:** Start with 1 ng – 1 µg of purified DNA.
- It is **important** to remove all cations and chelators from DNA preparations. If the DNA was dissolved in 1X TE, or you are not certain about the EDTA concentration in the input DNA, we strongly recommend purifying the input DNA using AMPure® XP beads, follow the instruction in Appendix I. Alternatively, we recommend applying the protocol in Appendix II for using input DNA in 1X TE buffer.
- It is **critical** to determine the input DNA concentration, especially when the input amount is below 100ng. We recommend using Qubit, Picogreen or another Fluorometric method to accurately quantify the DNA.

Make sure input DNA is in water, 10 mM Tris, Buffer EB or LoTE (0.1X TE)

If input DNA is in 1X TE, please purify the DNA follow the instruction in Appendix I, or use the protocol in Appendix III.

Protocol

5X WGS Fragmentation Mix

1. Before setting up the reaction, it is critical to determine the amount of the input DNA and the buffer the DNA is in. We strongly recommend users practicing the protocol and optimizing the reaction time using the same or similar DNA samples.

2. Prepare the following program into a thermal cycler (table below). Be certain to use the instrument's heated lid, and if possible, set the temperature of the heated lid to ~70°C. When the thermal cycler block reaches 4°C, pause the program.

The input DNA (1 - 1000 ng) is free of EDTA, or in EB or 0.1X TE

Step	Incubation Temperature	Incubation Time
1	4°C	1 min
2	32°C	3-24 min*
3	65°C	30 min
4	4°C	Hold

Guidelines for choosing the initial fragmentation Time

	Fragmentation Time (min) at 32°C			
Fragment Peak Size	250 bp	350 bp	450 bp	550 bp
10 ng input DNA	24	16	14	10
100 ng input DNA	16	10	8	6
1000 ng input DNA	14	8	6	4

*** Note:** The exact reaction time may need to be optimized for different amount of input DNA. The table above serves as a general guideline to help decide the starting point for optimization of the reaction time in order to achieve the desired fragment size. For initial optimization we recommend including 2 additional time points, one is 3 minutes longer and another one is 3 minutes shorter. Fine-tuning may be required if precise fragment size is critical. For more detailed guidance, please refer to Figure 1 in Appendix II.

For input DNA **≤10 ng**, to produce fragment size centers around 350 bp, we recommend adding **2.5 µl of Enhancer** in a 50 µl reaction and incubate for **10 min**.

3. It is important to follow the procedure described below, in order to achieve optimal results. The final reaction volume is 50 µl, and uses the table below to set up the reaction. Prepare a master mix on ice by combining Fragmentation Buffer, DNA, and nuclease-free water as indicated in the table (per DNA sample). Mix well by gently pipetting (**do not vortex to mix**). It can be scaled as needed for the desired number of samples (10% extra volume added to compensate for the pipetting loss when preparing the master mix for multiple samples).

For input DNA (> 10 ng)

	1 Reaction (µl)
10X Fragmentation Buffer	5
Purified DNA	X
Nuclease-free H_2O	(35- X)
Total	40

For input DNA (1 - 10 ng)

	1 Reaction (µl)
10X Fragmentation Buffer	5
Purified DNA	X
Enhancer	2.5
Nuclease-free H_2O	(32.5 - X)
Total	40

4. Transfer 10 µl of 5X WGS Fragmentation Mix to a new thin-walled PCR tube for each reaction. Add 40 µl of the master mix from step 3 and gently mix well by pipetting up and down 10-12 times. It is critical to keep the PCR tube on ice for the whole time during reaction setup.

5. Pulse-spin the sample tube and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.

6. When thermal cycler program is complete and sample block has returned to 4°C, remove samples from block and place on ice.

7. Immediately proceed to ligation step. To achieve optimal ligation efficiencies, we recommend using WGS Ligase (L6030-W-L) from Enzymatics.

Appendix I: Removal of Divalent Cations and EDTA from Input Nucleic Acid

Refer to manufacturer's protocol for details on methods of purification.

1. If DNA is in a volume of less than 50 µl, adjust the volume to 50 µl with nuclease-free water.
2. Add 90 µl of thoroughly vortexed AMPure® XP beads slurry to the reaction for a ratio of 1.8X and mix well by pipetting. If DNA is in a volume greater than 50 µl, scale the volume of AMPure® XP beads appropriately such that the ratio of beads to DNA is 1.8X.
3. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 2-4 min and carefully discard the supernatant without disturbing the beads.
4. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
5. Air-dry the beads on the magnetic stand for 10 min or until the beads are dry.
6. Thoroughly resuspend the dried beads in 45 µl of 10 mM Tris-HCl, pH 8.0. Pellet the beads on the magnetic stand for 2 min. Carefully transfer 42.5 µl of supernatant into a new tube.
7. Determine the concentration of the purified DNA using Quibit, Picogreen or other Fluorometric method.

Appendix II: Optimization of Fragmentation Time

The reaction time should be optimized for different input DNA (amount and source). Use the figure below to choose the time that is required to fragment the input DNA to the desired size. The optimization should be carried out using the same or similar DNA samples to what will be used in used for the final sequencing experiment. For initial optimization we recommend including 2 additional time points; 3 minutes longer and 3 minutes shorter than the time calculated from the figure. Fine-tuning may be required if precise fragment size is critical. Optionally, the fragmentation size can be evaluated immediately after step 1 if the input DNA amount is >10ng. Use 1.8X Ampure XP to purify the fragmented DNA and elute in 10 µl of Tris buffer or water, then use *Bioanalyzer High Sensitivity kit* to determine the size range of the fragmented DNA.

For input DNA ≤10 ng, to shorten the reaction time, we recommend adding 2.5 µl of Enhancer to the final reaction (50 µl) and use half of the reaction time determined from the fragmentation profile of 10 ng input DNA in the Figure 1. For example, to produce fragment size centers around 350 bp, after adding the Enhancer, a 10 minute incubation of the reaction usually generates expected result.

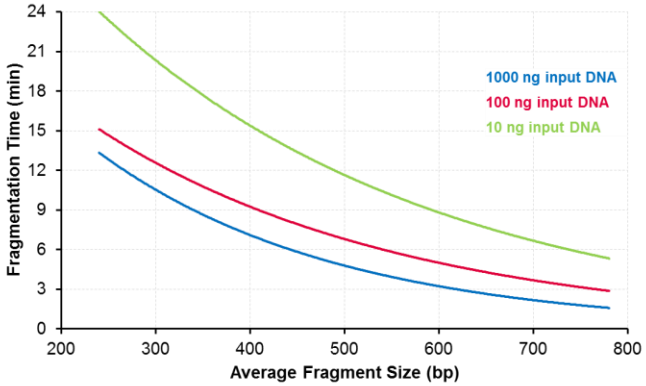

Figure 1: Fragmentation profile of different amount of input DNA

Appendix III: Fragmentation / End Repair/ dA-tailing of DNA in 1X TE

Follow the instructions below for input DNA in 1X TE buffer.

1. Enter the following program into a thermal cycler (Table below). Be certain to use the instrument's heated lid, and if possible, set the temperature of the heated lid to ~70°C.

Input DNA (1 - 1000 ng) in 1X TE

Step	Incubation Temperature	Incubation Time
1	4°C	1 min
2	32°C	5-35 min*
3	65°C	30 min
4	4°C	Hold

*** Note:** The reaction time should be optimized for different amount of input DNA. For input DNA >**10 ng**, we recommend **25 min as a starting point** as it produces fragmentation size centers around **300 to 500 bp**. For input DNA ≤**10 ng**, to produce fragment size centered around **300 bp**, we recommend **15 min**. Depending on the size requirement and type of input DNA, either increase or decrease reaction time by 3 min incrementally until expected size range is achieved. For more detailed guidance, please refer to Figure 2 below. For a direct comparison purpose, instead of 5 µl of Enhancer as suggested below, only 2.5 µl of Enhancer to the final reaction (50 µl) was used to generate the fragmentation profile of 10 ng input DNA in 1X TE.

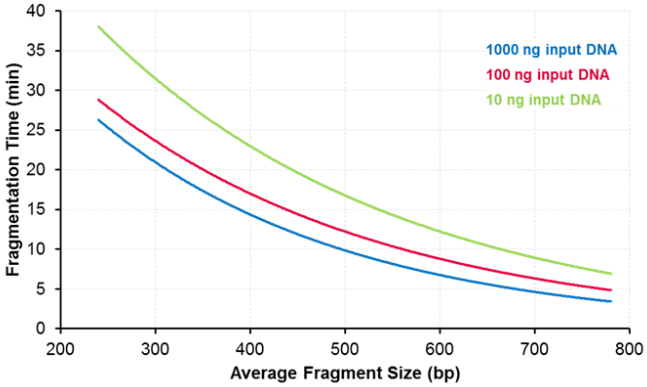

Figure 2: Fragmentation profile of different amount of input DNA in 1X TE

2. It is important to follow the procedure described below, in order to achieve optimal results. The final reaction volume is 50 µl, and uses the table below to set up the reaction. Prepare a master mix on ice by combining Fragmentation Buffer, DNA, and nuclease-free water as indicated in the table (per DNA sample). Mix well by gently pipetting (**do not vortex to mix**). It can be scaled as needed for the desired number of samples (10% extra volume added to compensate for the pipetting loss when preparing the master mix for multiple samples).

For input DNA (> 10 ng) in 1X TE

	1 reaction (µl)
10X Fragmentation Buffer	5
DNA in 1X TE	x
Enhancer	2.5
Nuclease-free H_2O	(32.5 - x)
Total	40

For input DNA (1-10 ng) in 1X TE

	1 reaction (µl)
10X Fragmentation Buffer	5
Purified DNA	x
Enhancer	5
Nuclease-free H_2O	(30 - x)
Total	40

3. Transfer 10 µl of 5X WGS Fragmentation Mix to a new thin-walled PCR tube for each reaction. Add 40 µl of the master mix and gently mix well by pipetting up-and-down 6-8 times. It is critical to keep the PCR tube on ice for the whole time during reaction setup.
4. Pulse-spin the sample tube and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.
5. When thermal cycler program is complete and sample block has returned to 4°C, remove samples from block and place on ice.
6. Immediately proceed to ligation step. To achieve optimal ligation efficiencies, we recommend using WGS Ligase (L6030-W-L) from Enzymatics for this step.

Quality Control

All kit components are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination. Detailed product information for individual kit components is available upon request.

Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.