TIANGEN®

TIANSeq Fragment/Repair/Tailing Module

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TIANSeq Fragment/Repair/Tailing Module

Cat.no. 4992350/4992351

Kit Contents

Contents	4992350 (24 rxn)	4992351 (96 rxn)
5×FEA Enzyme Mix	240 μl	960 μl
10×FEA Reaction Buffer	120 μl	480 μl
FEA Enhancer	120 μl	480 μl
Nuclease-Free ddH$_2$O	1 ml	4×1 ml
Handbook	1	1

Storage Conditions

TIANSeq Fragment/Repair/Tailing Module could be stored at -25°C~-15°C for 12 months. Avoid repeated freezing and thawing.

Product Description

TIANSeq Fragment/Repair/Tailing Module provides a pre-mix module of library construction for Illumina high-throughput sequencing platforms.

The kit includes all the enzymes for fragmentation, end-repair, and dA-tailing. The protocol supports fragmentation, end-repair and dA-tailing in a single reaction step, therefore greatly simplifying the workflow, reducing the total reaction time and hands-on time. And the product could be used directly for adapter ligation by TIANSeq Fast Ligation module(Cat# 4992354/4992355).

Application: ideal choice for the DNA fragmentation, end repairing and dA-tailing for DNA library construction of illumina high-throughput sequencing platform.

DNA input amount: 1 ng-1 μg DNA

Recommended Alternative Reagents

1. TIANseq Fast Ligation Module(Cat# 4992354/4992355)
2. TIANSeq NGS Library Amplification Module (Cat# 4992373/4992374)
3. TIANSeq Single-Indexed Adapter (Illumina)(Cat# 4992641/4992642/4992378)
4. TIANSeq Size Selection DNA beads(Cat# 4992358/4992359/4992979)

Product Highlights

1. The easily performed one-tube enzymatic reaction can achieve double-strand DNA fragmentation, end repair and dA-tailing in one step.
2. High library construction efficiency is achieved with the DNA input as low as 1 ng.

Precautions <u>Please carefully read these precautions before using this kit.</u>

1. Attention should be paid in the operating process to avoid cross contamination between nucleic acid samples and products.
2. Please use RNase- or DNase-free pipette tips or EP tubes for the experiment.
3. Before starting, wipe down work area with RNase and DNase cleaning reagents such as RNase Away (Molecular BioProducts, Inc). Make sure there is no contamination of RNase and DNase.
4. Before proceeding related operation, make sure the thermal cycler is calibrated and in a stable state.

5. Please read the protocol carefully before the experiment. If test suspension is needed or the downstream test is not needed to be carried out immediately, the test products can be frozen and stored at -20°C and the subsequent test can be planned accordingly.
6. 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.

Protocol:

1)Preparation:

1. Before the experiment, it is critical to determine the concentration of the input DNA and the buffer which DNA is dissolved in. DNA needs to be dissolved in the buffer as below: Deionized water, 10 mM Tris, Buffer EB or LoTE (0.1×TE).

   Note: If the DNA input amount is less than 100 ng, please use Qubit, Picogreen or other Fluorometric methods to accurately quantify the DNA. It is important to remove all cations and chelators from DNA. If the DNA was dissolved in 1×TE, or you are not certain about the EDTA concentration in the input DNA, we recommend purifying the input DNA using TIANSeq Size Selection DNA Beads (4992358/4992359/4992979), follow the instruction in Appendix I. Alternatively, we recommend applying the protocol in Appendix III for fragmentation.

2. Thaw reagents on ice. Once reagents are thawed, mix the 5×FEA enzyme Mix by finger flicking (do not vortex), and mix other components by quick vortex to avoid any localized concentrations.

2) Procedures

1. Set up the PCR program as below, and set the temperature of the heated lid to 70°C.

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

***Note: the exact fragmentation reaction time needs to be optimized based**

on the actual amount of input DNA sample. Table I below shows the reaction time to achieve the desired fragment size with 10 ng, 100 ng and 1,000 ng input DNA amount. Users can refer to the general guideline to optimized the reaction time. For the optimization, we recommend 2 additional time points as control, one with 3 min longer and the other with 3 min shorter. This can help to determining the exact reaction time required to achieve the desired fragment size. For more advices on fragmentation reaction time, please refer to Appendix II.

Table I: Guidelines for choosing fragmentation Time

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

2. Prepare a master mix on ice according to the table below. Mix well by gently pipetting (do not vortex to mix).

For input DNA≥10 ng	
Components	**Volume (μl)**
10×FEA Reaction Buffer	5
Purified DNA	X
Nuclease-Free ddH_2O	(35-X)
Total	40

For input DNA<10 ng	
Components	**Volume(μl)**
10×FEA Reaction Buffer	5
Purified DNA	X
FEA Enhancer	2.5
Nuclease-Free ddH_2O	(32.5-X)
Total	40

Note: for multiple reactions, calculate the total volume of reagents required and increase the volume by 10% to compensate for the pipetting loss, thus to ensure the solution is sufficient for desired numbers of samples.

3. Place a new 200 μl thin-walled tube on ice and add 10 μl 5×FEA Enzyme Mix to the tube. Then transfer 40 μl of the master mix in Step 2 to the same thin-walled tube and gently mix well by pipetting up and down for 10 times.
4. Pulse-spin the sample tube and immediately transfer to the pre-chilled thermal cycler (4°C). Resume the cycling program.
5. When the cycling program is completed, remove the thin-walled tube from block and put it on ice.
6. Immediately proceed to ligation step. To achieve optimal ligation efficiency, we recommend using TIANSeq Fast Ligation Module(Cat#4992354/4992355).

Appendix I: Removal of Divalent Cations and EDTA from DNA

It is recommended to use TIANSeq Size Selection DNA Beads (Cat# 4992358/ 4992359/4992979) for the purification of DNA. The steps are as follows:

1. Equilibrate the magnetic beads at room temperature for 20 min.
2. If DNA is dissolved in a buffer with the volume less than 50 μl, adjust the volume to 50 μl with Nuclease-Free ddH_2O.
3. Add 1.8× (90 μl) of thoroughly vortexed TIANSeq Size Selection DNA Beads to DNA solution and mix well by pipetting. If the volume of DNA solution is greater than 50 μl, scale the volume of TIANSeq Size Selection DNA Beads appropriately such that the ratio of beads to DNA is 1.8×.
4. Incubate the mixture for 5 min at room temperature. Pellet the beads on a magnetic stand for 5 min and carefully discard the supernatant without disturbing the beads.
5. Place the tube on the magnetic stand and add 200-500 μl freshly prepared 80% ethanol (the ethanol should be just enough to immerse all the beads) to the reaction tube, then gently pipette up and down for 3-5 times to wash the magnetic beads (do not disturb the beads). Pellet the magnetic beads with a magnetic stand for 30 sec and discard the supernatant.
6. Repeat the wash once.
7. Place the reaction tube on the magnetic stand, open the centrifuge tube cover and air-dry the beads at room temperature for 5-10 min until it is dried.
   Note: Do not over-dry the beads, otherwise the yield will be decrease.
8. Remove the reaction tube from the magnetic stand, and thoroughly resuspend the dried beads in 32.5 μl 10 mM Tris-HCl (pH8.0) with by pipetting up and down for 3-5 times. Place at room temperature for 5 min, then pellet the beads on the magnetic stand for 5 min. Carefully transfer 30 μl of supernatant into a new centrifuge tube after the magnetic beads are attached.

9.Determine the concentration of the purified DNA using Quibit, Picogreen or other Fluorometric methods.

Appendix II Optimization of Fragmentation Time

The reaction time should be optimized for different input DNA (amount and source). Use the Figure 1 to choose the time that is required to fragmentate the input DNA to the desired size. The optimization should be carried out using the same or similar DNA samples that will be 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 the fragmentation step if the input DNA amount is ≥100 ng. Use 1.8× TIANSeq Size Selection DNA Beads (Cat# 4992358/4992359/4992979) to purify the fragmented DNA and elute with 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 Table I. For example, to produce fragment size around 350 bp, after adding the FEA Enhancer, a 8 minute incubation of the reaction usually generates the expected result.

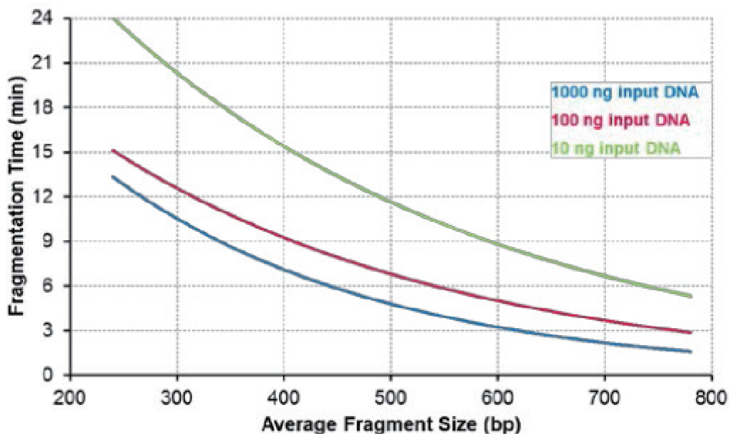

Figure 1 Fragmentation profile of different amounts of input DNA

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

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

1. Set up the program as 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		
Step	**Temperature**	**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, with 2.5 ul FEA Enhancer added in the reaction, we recommend 25 min as the initial time when it produces fragment size around 300 to 500 bp. For input DNA <10 ng, with 5 ul FEA Enhancer added in the reaction, we recommend 15 min as the incubation time to produce fragment size around 300 bp. Depending on the size requirement and type of input DNA, either increase or decrease the reaction time by 3 min incrementally until the expected size range is achieved.**

2. In order to achieve optimal results, it is important to follow the procedure described below. Prepare a master mix on ice by combining Fragmentation Buffer, DNA, and Nuclease-Free ddH_2O as indicated in the table (per DNA sample).Mix well by gently pipetting (do not vortex).

For input DNA≥10 ng	
Components	**Volume (μl)**
10×FEA Reaction Buffer	5
Purified DNA	X
FEA Enhancer	2.5
Nuclease-Free ddH_2O	(32.5-X)
Total	40

For input DNA<10 ng	
Components	**Volume (μl)**
10×FEA Reaction Buffer	5
Purified DNA	X
FEA Enhancer	5
Nuclease-Free ddH_2O	(30-X)
Total	40

Note: for multiple reactions, calculate the total volume of reagents required and increase the volume by 10% to compensate for the pipetting loss, thus to ensure the solution is sufficient for desired numbers of samples.

3. Transfer 10 μl of 5×FEA Enzyme 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 for 10 times. It is critical to keep the PCR tube on ice during the whole process.
4. Pulse-spin the sample tube and immediately transfer to the pre-chilled thermal cycler (4°C). Set up the program.
5. When thermal cycler program is complete and sample block has returned to 4°C, remove samples from the cycler and place on ice.
6. Immediately proceed to ligation step. To achieve optimal ligation efficiencies, we recommend using TIANSeq Fast Ligation Module (Cat# 4992354/ 4992355).