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TIANSeq DirectFast Library Kit (illumina)

Library Preparation Protocol with Fragmentation for Illumina Sequencing

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TIANSeq DirectFast Library Kit (illumina)

Cat. no. 4992259/4992260

Kit Contents

Contents	4992259 24 rxn	4992260 96 rxn
5×FEA Enzyme Mix	240 µl	960 µl
10×FEA Reaction Buffer	120 µl	480 µl
FEA Enhancer	120 µl	480 µl
TIANSeq DNA Ligase	240 µl	960 µl
5×Ligation Buffer	500 µl	2×1 ml
2×HiFi PCR MasterMix	600 µl	4×600 µl
P5/P7 Primers Mix (10 µM each)	120 µl	480 µl
Nuclease-Free ddH_2O	1 ml	4×1 ml
Handbook	1	1

Storage Conditions

The kit should be stored at -30~-15°C. Avoid repeated freezing and thawing. The shelf life is one year.

Product Description

The TIANSeq DirectFast Library Kit (illumina) is a DNA library construction kit specially optimized for illumina high-throughput sequencing platform. The kit is designed to perform DNA fragmentation, end repair and 3'-end dA-tailing in one step within a single tube. The product after this step can be directly used for adapter ligation without additional purification step. In addition, the PCR amplification reagent in this kit has been specially optimized to ensure high yield, good fidelity and no base preference for the amplified DNA library. The one-step streamline workflow of this kit skips multiple purification steps, and make sure the entire library construction process can be performed in 2.5 hr. This kit can achieve higher library construction efficiency, and can ensure the high efficiency of library construction for low input DNA samples.

Applications: ideal choice for the DNA library construction for illumina high-throughput sequencing platform.

Sample input amounts: 1 ng-1 μg DNA.

Other Recommended Reagents

1. TIANSeq Single-Index Adapter (illumina)(Cat# 4992641/4992642/4992378).
2. TIANSeq Size Selection DNA Beads(Cat# 4992358/4992359/4992979).

Product Highlights

1. No base preference in the process of DNA fragmentation and PCR amplification, and the sequencing uniformity is ensured.
2. High library construction efficiency can be achieved even with the DNA input as low as 1 ng.
3. The easily performed one-tube enzymatic reaction skips multiple purification steps, and shortens the entire library construction process to only 2.5 hr.

Precautions **Please carefully read these precautions before using this kit.**

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 experiments.
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 the library amplification, 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, reaction time, setup conditions, as well as the input DNA amount. It is highly recommended that users practice the experiment according to the steps and the optimized reaction parameters (such as reaction time) described in the protocol.

Protocol

I. Fragmentation/End repair/dA-tailing

(I) Preparation:

1. Before the experiment, it is critical to determine the concentration of the input DNA and the buffer which DNA is dissolved in.

   Note: it is critical to determine the quality and concentration of the input DNA sample, especially when the input amount is below 100 ng. Qubit, Picogreen or other Fluorometric methods are recommended for accurately quantifying the DNA. In addition, please confirm the buffer in which the DNA is dissolved, as the protocol needs to be slightly adjusted with different buffers.

2. Place the reagents on ice. Once thawed, mix the 5× FEA Enzyme Mix by gentle finger flicking (do not vortex to mix). The remaining reagents can be mixed by quick vortexing.

(II) Procedures

1. If DNA is dissolved in deionized water, 10 mM Tris, Buffer EB or 0.1× TE, set up the fragmentation/end repair/dA-tailing reaction following the steps below.

   (1) Prepare the following program (table below) into a thermal cycler. Turn on the hot lid and set the temperature to 70°C.

Reaction Step	ReactionTemperature	Reaction 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 1 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 the instructions of TIANSeq Fragment/Repair/Tailing Module (4992350/4992351).**

Table 1 Guidelines for Fragmentation Time Selection

Fragment Peak Size	Fragmentation Time (min) (32°C)			
	250 bp	350 bp	450 bp	550 bp
10 ng input DNA	24	16	14	10
100 ng input DNA	16	10	8	6
1,000 ng input DNA	14	8	6	4

(2)Prepare the reaction mix on ice according to the following table. Mix well by gently pipetting. Do not vortex to mix.

Components	Volume (μl)	
	Input DNA≥10ng	Input DNA<10ng
10×FEA Reaction Buffer	5	5
DNA sample	X	X
FEA Enhancer	0	2.5
Nuclease-Free ddH_2O	(35-X)	(32.5-X)
Total volume	40	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 thin-walled tube, and immediately put it in the thermal cycler that has been pre-cooled to 4°C, then start the cycling program.

(5)When the cycling program is completed, remove the thin-walled tube from block and put it on ice.

(6)Proceed to the adapter ligation step immediately.

2. If DNA is dissolved in 1× TE buffer, please refer to the following steps for DNA fragmentation/end repair/dA-tailing reaction.

(1)Prepare the following program (table below) into a thermal cycler. Turn on the hot lid and set the temperature to 70°C.

Reaction Step	Reaction Temperature	Reaction Time
1	4°C	1 min
2	32°C	5-35 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. For input DNA amount ≥10 ng, add 2.5 µl FEA Enhancer to the reaction mix. 25 min is recommended as the initial reaction time. At this point, the product fragment size centers around 300-500 bp. For input DNA amount <10 ng, add 5 µl FEA Enhancer to the reaction mix. 15 min is recommended as the initial incubation time. At this point, the product fragment size centers around 300 bp. If adjustment is required, a 3-min's increase or decrease shall be conducted based on the initial reaction time recommended above, until the desired fragment size is obtained.**

(2)Prepare the reaction mix on ice according to the following table. Mix well by gently pipetting. Do not vortex to mix

Components	Volume (μl)	
	Input DNA≥10ng	Input DNA <10ng
10×FEA Reaction Buffer	5	5
DNA sample	X	X
FEA Enhancer	2.5	5
Nuclease-Free ddH_2O	(32.5-X)	(30-X)
Total volume	40	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 thin-walled tube, and immediately put it in the thermal cycler that has been pre-cooled to 4°C, then start the cycling program.

(5)When the cycling program is completed, remove the thin-walled tube from block and put it on ice.

(6)Proceed to the adapter ligation step immediately.

3. If DNA is dissolved in other solutions, determine the concentration of saline ions in the solution, especially EDTA. EDTA has an apparent influence on the reaction. If the concentration of EDTA in the solution is uncertain or is in a high level, we recommend applying TIANSeq Size Selection DNA Beads (Cat# 4992358/4992359/4992979) for the purification of DNA. The purification steps are as follows:

(1)Equilibrate the magnetic beads at room temperature for 20 min.

(2)If the volume of DNA solution is less than 50 μl, adjust the volume to 50 μl with nuclease-free deionized water.

(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)After incubating at room temperature for 5 min, place the reaction tube on the magnetic stand for 5 min to pellet the magnetic beads,

and carefully remove the supernatant by a pipette.

(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 this washing step 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 the magnetic beads are 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)Use Quibit, Picogreen or other Fluorometric methods to determine the concentration of the purified DNA.

II. Adapter Ligation

Place the reagents on ice. Once thawed, mix the TIANSeq DNA Ligase by gentle finger flicking (do not vortex to mix). The remaining reagents can be mixed by quick vortexing.

1. After the fragmentation/end repair/dA-tailing reaction, add Y µl adapter solution to the 50 µl reaction mix, mix gently by pipetting and then put on ice.

   Notes: This kit does not contain the DNA adapter for sequencing. Please refer to the usage conditions provided by the adapter supplier. TIANSeq Single-Index Adapter (illumina)(4992641/4992642/4992378) is recommended. To achieve higher ligation efficiency, we recommend the molar ratio of the adapter to the DNA fragments in the reaction mix to be between 10:1 to 200:1.

2. Prepare the reaction master mix according to the table below. Mix gently by pipetting and then keep it on ice.

Components	Volume (µl)
5×Ligation Buffer	20
TIANSeq DNA Ligase	10
Nuclease-Free ddH_2O	(20-Y)
Total volume	(50-Y)

3. Add the prepared (50-Y) µl ligation master mix to the reaction solution in Step 1 and mix well by gently pipetting up and down for 10 times. Incubate the ligation reaction at 20°C for 15 min.
   Notes: if this step is performed using a thermal cycler, set the hot lid temperature to ≤40°C.
4. It is recommended to use 1× (100 µl) TIANSeq Size Selection DNA Beads (4992358/4992359/4992979) for the purification of ligation products. The steps are as follows:
   (1)Equilibrate the magnetic beads at room temperature for 20 min.
   (2)Vortex the magnetic beads to full suspension. Add 100 µl of the thoroughly vortexed the magnetic beads to the solution in Step II/3, and mix well by pipetting thoroughly for 10 times.
   (3)Incubate the mix for 5 min at room temperature. Place the reaction tube on the magnetic stand for 5 min. After the magnetic beads are completely attached, carefully remove the supernatant by a pipette.
   (4)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.
   (5)Repeat the washing step once.
   (6)Place the reaction tube containing magnetic beads on the magnetic stand, open the lid and keep it at room temperature for 5-10 min until it is dried.
   Note: do not over-dry magnetic beads, as this will cause a decrease in the yield.
   (7)Remove the reaction tube from the magnetic stand, and thoroughly resuspend the magnetic beads by adding 22.5 µl 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and gently mix by pipetting up and down for 10 times. Place the tube at room temperature for 5 min, then put the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, transfer about 20 µl of the supernatant to a new centrifuge tube for subsequent PCR amplification.
   Note: alternatively, if library amplification is not intended for the ligation product, add 12.5 µl of 10mM Tris-HCl (pH 8.0) to Step (6) for the elution of DNA, and 10 µl of the purified DNA can be transferred for subsequent application. If not proceeding immediately, please keep samples stored at -20°C.
   (8)If size selection is needed, use 102.5 µl nuclease-free ddH_2O for beads elution. Transfer 100 µl supernatant to a new centrifuge tube for subsequent size selection.

5. For size selection: TIANSeq Size Selection DNA Beads (Cat# 4992358/4992359/4992979) is recommended. Please refer to the proportion of magnetic beads in the size selection process in Table 2 for operation. If other magnetic beads are applied, please refer to the protocols of the corresponding magnetic beads.

Table 2 Recommended Magnetic Beads amount for size selection

Library Parameters		Magnetic Beads Addition Ratio	
Initial Fragment Size	Fragment Size After Adapter ligation	First Selection Ratio	Second Selection Ratio
250 bp	370 bp	0.6×	0.1×
300 bp	420 bp	0.55×	0.1×
350 bp	470 bp	0.53×	0.1×
400 bp	520 bp	0.5×	0.1×
450 bp	570 bp	0.47×	0.1×
500 bp	620 bp	0.45×	0.1×

Taking the case with the initial fragment size of 250 bp as an example, when TIANSeq Size Selection DNA Beads are used for purification, the specific steps are as follows:

(1)Equilibrate the magnetic beads at room temperature for 20 min.

(2)Vortex the magnetic beads to full suspension. Add 0.6× (60 μl) magnetic beads (according to the first selection ratio in Table 2) to 100 μl ligation product from Step 4(8).

(3)Incubate the mix for 5 min at room temperature. Place the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, carefully transfer the supernatant to a new centrifuge tube containing 0.1×(10 μl) magnetic beads with a pipette (do not aspirate the beads when pipetting), and immediately pipette up and down for at least 10 times to mix thoroughly.

(4)Incubate the mix for 5 min at room temperature. Place the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, carefully remove the supernatant with a pipette.

(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 washing step once.

(7)Place the reaction tube containing the magnetic beads on the magnetic stand, open the lid and keep it at room temperature for 5-10 min until it is dried.

Note: do not over-dry magnetic beads, as this will cause a decrease in the yield.

(8)Remove the reaction tube from the magnetic stand, and thoroughly resuspend the magnetic beads by adding 22.5 µl 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and gently mix by pipetting up and down for 10 times. Leave at room temperature for 5 min, and keep the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, transfer about 20 µl of the supernatant to a new centrifuge tube for subsequent PCR amplification.

III. Library Amplification

1. Thaw the 2× HiFi PCR MasterMix and P5/P7 Primers Mix (10 µM each) on ice. Once thawed, mix the 2× HiFi PCR MasterMix by finger flicking and inverting up and down. The P5/P7 Primer Mix (10 µM) can be mixed by quick vortexing.

2.Program a thermal cycler with the parameters listed in the table below. Turn on the hot lid and set the temperature to 105°C.

Step	Temperature	Time	Cycle numbers
1	98°C	2 min	1
2	98°C	20 sec	6-12*
3	60°C	30 sec	
4	72°C	30 sec	
5	72°C	1 min	1
6	4°C	Hold	1

* **Note: please determine the PCR cycle numbers according to the DNA input amount. Generally, for 100 ng, 10 ng and 1 ng input library DNA, the recommended PCR amplification cycle numbers are 6, 10 and 12, respectively. If the fragment size selection step has been carried out before PCR amplification, it is recommended that additional 2-4 cycles should be added. If the quality of DNA is poor (for example, extracted from FFPE samples), it is recommended that additional 1-3 cycles should be added.**

3. Prepare the PCR reaction according to the following table. Note that this step needs to be operated on ice.

Components	Volume (µl)
2×HiFi PCR MasterMix	25
P5/P7 Primers Mix(10 µM each)	5
Total volume	30

4. Transfer 20 µl of the purified ligation product to the PCR tube. Add 30 µl PCR reaction solution prepared in Step 3 into the tube and gently mix by pipetting for 10 times.

   Note: Please keep the reaction tube on ice the whole time during reaction set up.

5. Pulse-spin the PCR reaction tube, and immediately transfer the tube to the thermal cycler. Start the program that's been set in Step 2.

6. When the thermal cycler program is complete and sample block has returned to 4°C , remove the sample from the block and proceed to the purification step using 1× (50 µl) TIANSeq Size Selection DNA Beads (Cat# 4992358/4992359/4992979).

   (1)Equilibrate the magnetic beads at room temperature for 20 min.

   (2)Vortex the magnetic beads to full suspension. Add 50 µl magnetic beads to the solution. Mix thoroughly by pipetting up and down for 10 times.

   (3)Incubate the mix for 5 min at room temperature. Place the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached, carefully remove the supernatant with a pipette.

   (4)Place the reaction tube on the magnetic stand. 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.

   (5)Repeat the washing in Step (4) once.

   (6)Place the reaction tube containing the magnetic beads on the magnetic stand. Open the lid to dry for 5-10 min at room temperature until it is dried.

   Note: Do not over-dry magnetic beads, as this will cause a decrease in the yield.

   (7)Remove the reaction tube from the magnetic stand, and thoroughly resuspend the magnetic beads by adding 22.5 µl of 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and mix the magnetic beads thoroughly by pipetting up and down for 10 times. Place at room temperature for 5 min, and keep the reaction tube on the magnetic stand for 5 min. When the magnetic beads are completely attached to the tube wall, transfer 20 µl of the supernatant to a new centrifuge tube.

7. The quality of the DNA library can be evaluated by gel electrophoresis, qPCR quantification or Agilent bioanalyzer before sending for sequencing. The purified DNA library can be stored at -20°C.