TIANSeq Fast RNA Library Kit (illumina)

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Cat. no. 4992375/4992376

Kit Contents

Contents	4992375 (24 rxn)	4992376 (96 rxn)
Frag/1st Strand Buffer	120 µl	480 µl
1st Strand Enzyme Mix	40 µl	160 µl
2nd Strand Buffer	240 µl	960 µl
2nd Strand Enzyme Mix	90 µl	360 µl
10×ERA Buffer	120 µl	480 µl
5×ERA Enzyme Mix	240 µl	960 µl
TIANSeq DNA Ligase	240 µl	960 µl
5×Ligation Buffer	500 µl	2×1 ml
2×HiFi PCR Master Mix	600 µl	4×600 µl
P5/P7 Primers Mix	120 µl	480 µl
Nuclease-Free ddH_2O	2×1 ml	8×1 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 Fast RNA Library Kit (Illumina) is a non-directional transcriptome library construction kit specialized developed for the Illumina high-throughput sequencing platform. The kit adopts a rapid one-tube protocol for rapid library construction of RNA samples. After double-stranded cDNA synthesis, the end-repair and dA-tailing can be completed in one step. The resulting product can be directly used for the adapter ligation without purification. In addition, the kit provides a specially designed high-efficiency high-fidelity polymerase, which ensures the obtained PCR-enriched product with high fidelity and no base preference.

The suitable input samples are total RNA with rRNA removed (containing mRNA and other non-coding RNAs), or mRNA isolated directly from total RNA. The input amount of the total RNA sample is 10 ng-1 μg; and the input amount of the mRNA sample can be as low as 500 pg.

Applications: Suitable for the construction of RNA library for Illumina high-throughput sequencing platform.

Sample input amount: 10 ng-1 μg of total RNA; For animal, plant and fungal mRNA, as low as 500 pg.

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. This kit can be used to perform transcriptome analysis on mRNA and non-coding RNAs (rRNA excluded), such as lncRNA.
2. The operation process is simple and can be used for rapid library construction of RNA samples.
3. High library conversion rate. Suitable for efficient library conversion of low input samples.
4. The fidelity of PCR amplification is high with no base preference.

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 and 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. Please read the instructions carefully before the experiment. The experiment can be paused at certain steps and samples can be stored according to the institutions.
5. Use high-quality RNA samples with RIN value ≥ 7.0 and good integrity for rRNA removal or mRNA isolation, otherwise the quality of the RNA library will be affected.

Protocol

I. RNA Fragmentation and Priming

1. Preparation:

(1) Take out the total RNA with rRNA removed or mRNA samples from the -80°C freezer and place on ice to slowly thaw the samples.

(2) Before starting the experiment, make sure the input amount of the total RNA with rRNA removed or mRNA is within 1-100 ng.

Note: It is important to determine the input amount of the total RNA with rRNA removed or mRNA. It is recommended to use Agilent 2100 to analyze the quality and concentration of the samples. The rRNA residue should be controlled within 10% to avoid its effect on the data analysis quality after library construction.

2. Procedures

Take out the Frag/1st Strand Buffer from the -20°C fridge and thaw on ice. Mix by votexting, and set up the following reaction in a PCR tube. Gently pipette up and down for 10 times to mix thoroughly. Place the sample tube in the thermal cycler and choose the reaction conditions for fragmentation according to the sizes of the insert:

(1) Prepare the following program (table below):

Components	Volume(μl)
Total RNA (rRNA removed) or mRNA	5
Frag/1st Strand Buffer	5
Total	10

(2) Fragmentation conditions for different insert sizes:

Insert sizes(bp)	Temperature	Time
150-200	94°C	15 min, 4°C hold
200-300	94°C	10 min, 4°C hold
300-400	94°C	6 min, 4°C hold
400-500	94°C	5 min, 4°C hold

Note: If the insert size range is within 150-200 bp, no size selection is needed for the subsequent experiments, and the RNA library with a relatively narrow peak in the expected size range can be achieved. For insert size larger than 200 bp, the size selection step is needed. Please refer to the size selection step below.

After the above reaction, quickly place the products on ice, and immediately proceed to the first strand cDNA synthesis step. Do not stop at the fragmentation and first strand cDNA synthesis steps, since RNA is easily degraded under this system.

II. First Strand cDNA Synthesis

1. Take out the 1st Strand Enzyme Mix from the -20°C fridge and mix by gently flicking. Set up the following reaction system in a PCR tube. Pipette up and down for 10 times to mix thoroughly:

Components	Volume(μl)
Fragmented RNA	10
1st Strand Enzyme Mix	1.5
Nuclease-Free ddH_2O	8.5
Total	20

Note: If multiple sample reactions are performed simultaneously, a mixture of 1st Strand Enzyme and Nuclease-Free ddH_2O can be prepared in advance in a centrifuge tube. The mixture can then be

dispensed into each reaction tube. It is recommended to prepare 1.1x the volume of the actual reaction mixture.

2.Perform the first strand cDNA synthesis reaction in a thermal cycler. Turn on the hot lid and set the temperature to 80°C:

Step	Temperature	Time
1	25°C	10 min
2	42°C	15 min
3	70°C	15 min
4	4°C	hold

Note: Immediately proceed to the second strand cDNA synthesis after the reaction.

III. Second Strand cDNA Synthesis

1. Take out the 2nd Strand Buffer and 2nd Strand Enzyme Mix from the -20°C fridge and thaw on ice. Mix the 2nd Strand Enzyme Mix by gently finger flicking (do not vortex). Other reagents can be mixed by quick vortexing. Set up the following reaction system in a PCR tube. Pipette up and down for 10 times to mix thoroughly:

Components	Volume(μl)
1st st Strand cDNA	20
2nd Strand Buffer	8.5
2nd Strand Enzyme Mix	3.5
Nuclease-Free ddH_2O	48
Total	80

2. Perform the second strand cDNA synthesis reaction in a thermal cycler. Turn on the hot lid and set the temperature to ≤40°C:

Step	Temperature	Time
1	16°C	60 min
2	4°C	hold

Note: After the reaction, the synthesized second strand cDNA can be temporarily stored at 4°C for 1 hour, but it is recommended to proceed to the next purification step immediately.

IV. Double-stranded cDNA Purification

Purify the double-stranded cDNA by adding 1.8x (144 μl) TIANSeq Size Selection DNA Beads (Cat# 4992358/4992359/4992979) to the second strand synthesis reaction (80 μl) in setp III 2. Please see the steps follow:

1. Equilibrate the beads for 20 min at room temperature.

2. Vortex the magnetic beads to full suspension. Add 144 µl of magnetic beads to the double-stranded cDNA product from step III 2, and gently pipette up and down for 10 times to thoroughly mix the reaction.

3. Incubate for 5 min at room temperature to allow DNA binding to the beads. Place the reaction tube on a magnetic stand for about 5 minutes. After the magnetic beads are completely attached, carefully remove and discard the supernatant with 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 step 4 once.

6. Keep the reaction tube on the magnetic stand, and air-dry the magnetic beads at room temperature for 5-10 min until the magnetic beads are dried.

Note: Do not disturb the magnetic beads when rinsing the magnetic beads with 80% ethanol. Pulse-spin the tube after the two washing steps and use a pipette to remove the liquid residual after the washings. Do not over-dry the beads, as this will cause cracking and reduce recovery efficiency.

7. Remove the reaction tube from the magnetic stand, and add 37.5 µl of Nuclease-free ddH_2O to the centrifuge tube and gently pipette the beads up and down for 10 times to a full suspension. Incubate the beads at room temperature for 5 min, then place the reaction tube on the magnetic stand for 5 min. After the magnetic beads are completely attached, transfer 35 µl of the supernatant to a new tube. The product can be used for subsequent experiments.

Note: Do not aspirate the magnetic beads when transferring the supernatant, as this will affect the quality of the library. The purified product in this step can be stored at -20°C.

V. End Repair/ dA-Tailing

1. Take out 10×ERA Buffer and 5×ERA Enzyme Mix from the -20°C fridge and thaw on ice. Mix the 5×ERA Enzyme Mix by gently flicking the tube (do not vortex). Other reagents can be mixed by quick vortexing. Set up the following reaction system in a PCR tube and mix thoroughly with by pipetting up and down for 10 times:

Components	Volume(μl)
cDNA	35
10×ERA buffer	5
5×ERA Enzyme Mix	10
Total	50

Note: This step needs to be performed in the ice bath. If multiple sample reactions are performed at the same time, a mixture of 10x ERA buffer and 5x ERA Enzyme can be prepared in advance in a centrifuge tube. The mixture can then be dispensed into each reaction tube. It is recommended to prepare 1.1x the volume of the actual reaction mixture.

2. Perform the following reaction in a thermal cycler. Set the hot lid temperature to 70°C.

Step	Temperature	Time
1	4°C	1 min
2	20°C	30 min
3	65°C	30 min
4	4°C	hold

3. When the reaction program is completed, place the reaction product on ice and immediately proceed to the adapter ligation step.

VI. Adapter Ligation

1. Take out the Adapter, 5×Ligase Buffer and TIANSeq DNA Ligase from the -20°C fridge and thaw on ice. Mix the TIANSeq DNA Ligase well by gently flicking (do not vortex). Other reagents can be mixed by quick vortexing. Dilute the adapters to corresponding concentrations according to the below table.

Total RNA(ng)	Adapter Concentration
1000 - 250	1.5 μM
249 - 100	300 nM
99 - 10	75 nM

Note: For bacterial RNA, because of the difference in RNA expression of

different strains, the adapters can be adjusted to lower concentrations based on the table above to avoid the forming of adapter dimers.

2. Set up the following reaction system in a PCR tube according to the following table, and mix thoroughly with by pipetting up and down for 10 times:

Components	Volume(μl)
dA-Tailing product	50
Adapter	5
5×Ligation Buffer	20
TIANSeq DNA Ligase	10
Nuclease-Free ddH_2O	15
Total	100

Note: This kit does not contain the sequencing adapters. TIANSeq Single-Index Adapter (Illumina) (4992641/4992642/4992378) is recommended for the adapter ligation. Please see the product manual for details. Add the diluted adapters with the corresponding concentrations to the reaction system for different RNA input amounts. The adapter ligation step needs to be performed in the ice bath. If multiple sample reactions are performed at the same time, a mixture of 5x Ligase Ligation Buffer, TIANSeq DNA Ligase and Nuclease-Free ddH_2O can be prepared in advance in a centrifuge tube. The mixture can then be dispensed into each reaction tube. It is recommended to prepare 1.1x the volume of the actual reaction mixture.

3. Perform the following reaction in a thermal cycler. Turn on the hot lid and set the temperature ≤ 40°C.

Step	Temperature	Time
1	20°C	15 min
2	4°C	hold

4. Purification of the adapter ligation product and size selection

This step provides two alternative options. Option (1) does not require the size selection step after one round of magnetic beads purification, and is suitable for the library construction with the insert size at the range of 150-200 bp. The fragments with the sizes 150-200 bp can be effectively obtained, and the adapter residue can be removed by performing the fragmentation at 94°C for 15 min. Option (2) requires two rounds of size selection after the magnetic beads purification step. By choosing different size selection conditions, different inserts with the sizes more than 200 bp can be obtained and the adapter residue can be removed. TIANSeq Size Selection DNA Beads (4992358/4992359/4992979) is recommended for the size selection.

Option (1): For the RNA library with the insert sizes at the range of 150-200 bp, the purification steps are as follows:

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 beads for 20 min at room temperature.

(2) Vortex to fully suspend the magnetic beads. Add 100 μl magnetic beads to the adapter ligation product solution, and gently pipette up and down for 10 times to thoroughly mix the beads.

(3) Incubate for 5 min at room temperature to allow DNA binding to the beads. Place the reaction tube on a magnetic stand for 5 minutes. After the magnetic beads are completely attached, carefully discard the supernatant with 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 step (4) 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 disturb the magnetic beads when rinsing the magnetic beads with 80% ethanol. Pulse-spin the tube after the two washing steps and remove the liquid residue as much as possible by a pipette after the washings. Do not over-dry the beads, as this will cause cracking and reduce recovery efficiency.

(7)Remove the reaction tube from the magnetic stand, and add 22.5 μl of 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and gently pipette the beads up and down for 10 times to the full suspension. Incubate at room temperature for 5 min, then place the reaction tube on a magnetic stand for 5 min. After the magnetic beads are completely attached, transfer 20 μl of the supernatant to a new centrifuge tube for subsequent PCR amplification experiments.

Note: Do not aspirate the magnetic beads when transferring the supernatant, as this will affect the quality of the subsequent library.

Option (2): For RNA library with the insert sizes more than 200 bp, the purification steps are as follows:

1. Purify the ligation product by the following steps:

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 beads for 20 min at room temperature.

(2) Vortex to fully suspend the magnetic beads, add 100 μl of magnetic beads to the adapter ligation product solution, and gently pipette up and down for 10 times to mix thoroughly.

(3) Incubate for 5 min at room temperature to allow DNA binding to the beads. Keep the reaction tube on the magnetic stand for 5 minutes. After the magnetic beads are completely attached, carefully discard the supernatant with 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 step (4) 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 disturb the magnetic beads when rinsing the magnetic beads with 80% ethanol. Pulse-spin the tube after the two washing steps and remove the liquid residue as much as possible by a pipette after the washings. Do not over-dry the beads, as this will cause cracking and reduce recovery efficiency.

(7)Remove the reaction tube from the magnetic stand, and add 102.5 μl of Nuclease-Free ddH_2O to the centrifuge tube and gently pipette the beads up and down for 10 times to full suspension. Incubate at room temperature for 5 min, then place the reaction tube on the magnetic stand for 5 min. After the magnetic beads are completely attached, transfer 100 μl of the supernatant to a new tube.

Note: Do not aspirate the magnetic beads when transferring the supernatant, as this will affect the quality of the RNA library.

2. For the two-round size selection (take the insert sizes at the range of 200-300 bp as an example. For other insert sizes, please select the corresponding volume of magnetic beads according to Table 1), the procedure is as follows:

Table 1 Recommended Size Selection Conditions for Different Insert Sizes

Insert Size(bp)	200-300	300-400	400-500
Library fragment size(bp)	320-420	420-520	520-620
Fragmentation condition	94°C-10min	94°C-6min	94°C-5min
Beads volume for first selection	0.6×	0.57×	0.47×
Beads volume for second selection	0.1×	0.1×	0.1×

It is recommended to use 0.6× (60 μl) TIANSeq Size Selection DNA Beads

(4992358/4992359/4992979) for the purification of ligation products. The steps are as follows:

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

(2) Vortex to fully suspend the magnetic beads, add 60 μl of magnetic beads to the purified adapter ligation product solution, gently pipette up and down for 10 times to mix thoroughly.

(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. Be careful not to aspirate the beads when transferring the supernatant.

(4) Incubate the tube at room temperature to make the DNA fully bind to the magnetic beads. Keep the reaction tube on the magnetic stand for about 5 minutes. After the magnetic beads are completely attached, pipette carefully to remove the supernatant.

(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 step (5) once.

(7)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 disturb the magnetic beads when rinsing the magnetic beads with 80% ethanol. Pulse-spin the tube after the two washing steps and remove the liquid residue as much as possible by a pipette after the washings. Do not over-dry the beads, as this will cause cracking and reduce recovery efficiency.

(8) Remove the reaction tube from the magnetic stand, and add 22.5 μl of 10 mM Tris-HCl (pH8.0) to the centrifuge tube and gently pipette the beads up and down for 10 times to full suspension. Incubate at room temperature for 5 min, then place the reaction tube on a magnetic stand for 5 min. After the magnetic beads are completely attached, transfer 20 μl of the supernatant to a new centrifuge tube for subsequent PCR amplification experiments.

Note: Do not aspirate the magnetic beads when transferring the supernatant, as this will affect the quality of the RNA library. The purified product in this step can be stored at -20°C.

VII. Library Amplification

1. Take out the 2×HiFi PCR Master Mix and P5/P7 Primers Mix from the -20°C fridge and thaw on ice. Once thawed, mix the 2× HiFi PCR MasterMix by finger flicking and inverting up and down (do not vortex). Other reagents can be mixed by quick vortexing. Set up the following reaction system in a PCR tube and mix thoroughly by pipetting up and down for 10 times:

Components	Volume(μl)
Purified adapter ligation product	20
2×HiFi PCR Master Mix	25
P5/P7 Primers Mix	5
Total	50

2. The library enrichment reaction is performed in a thermal cycler, with the hot lid temperature set to 105°C:

Step	Temperature	Time	Cycle numbers
1	98°C	2 min	1
2	98°C	20 sec	8-16*
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 based on the input amount and quality of total RNA samples (RIN≥7.0). After the size selection step, amplify for 10-12 PCR cycles for 1000 ng input total RNA, and 13-14 cycles for 100 ng input total RNA, and 15-16 cycles for 10 ng input total RNA. Decrease 1-2 cycles if size selection is not applied.**

3. Purification of PCR Reaction Product

It is recommended to use 1× (100 μl) TIANSeq Size Selection DNA Beads (4992358/4992359/4992979) for the purification of PCR products. The steps are as follows:

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

(2) Vortex to fully suspend the magnetic beads. Add 50 μl of magnetic beads to the PCR product solution, and gently pipette up and down for 10 times to mix thoroughly.

(3) Incubate for 5 min at room temperature to allow DNA binding to the beads. Place the reaction tube on a magnetic stand for about 5 minutes. After the magnetic beads are completely attached, carefully discard the supernatant.

(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 step (4) 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.

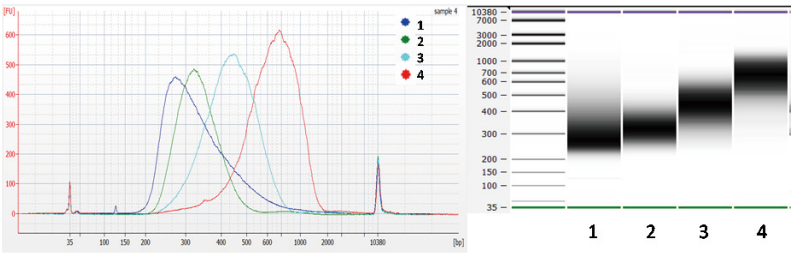
Note: Do not disturb the magnetic beads when rinsing the magnetic beads with 80% ethanol. Pulse-spin the tube after the two washing steps and remove the liquid residue as much as possible by a pipette after the washings. Do not over-dry the beads, as this will cause cracking and reduce recovery efficiency.

(7) Remove the reaction tube from the magnetic stand, and add 22.5 µl of 10 mM Tris-HCl (pH 8.0) to the centrifuge tube and gently pipette the beads up and down for 10 times to full suspension. Incubate at room temperature for 5 min, then place the reaction tube on the magnetic stand for 5 min. After the magnetic beads are completely attached, transfer 20 µl of supernatant to a new tube.

Note: Do not aspirate the magnetic beads when transferring the supernatant, as this will affect the quality of the RNA library.

4. Evaluation the library quality by Agilent 2100 Bioanalyzer (Agilent High Sensitivity Chip)

Dilute the obtained RNA library appropriately and load 1 µl for Agilent 2100 Bioanalyzer analysis (Agilent High Sensitivity Chip). A high-quality library will result in a concentrated peak within the expected size range, as shown in Figure 1. If a peak appears at around 120 bp, it is suggested that the library contains adapter-dimer contamination. The contamination can be effectively removed by diluting the library to 50 µl with Nuclease-free ddH_2O and repeating the PCR product purification step.



Library sizes:1.270-320bp; 2.320-420bp; 3.420-570bp; 4.570-670bp

Figure 1. 200 ng Rat reference RNA were fragmented under 4 different conditions, and size-selected by different selection ratios listed in Table 1