TIANGEN®

TIANSeq RNA Frag/cDNA Synthesis Module

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Cat. No. 4993005/4993006

Kit Contents

Contents	4993005 (24 rxn)	4993006 (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
Nuclease-Free ddH_2O	2×1 ml	8×1 ml
Handbook	1	1

Storage Conditions

Please store the kit at -15 ~ -25°C and avoid repeated freezing and thawing. The shelf life is one year.

Introduction

The TIANSeq RNA Frag/cDNA Synthesis Module is a module of RNA fragmentation and cDNA synthesis in the non-directional RNA library construction under the Illumina high-throughput sequencing platform, which can conduct synthetic reaction of double-strand cDNA. The input sample suitable for this kit can be both the rRNA-removed total RNA, and the mRNA directly isolated from total RNAs. This kit includes a complete set of enzymes and reaction buffers for RNA fragmentation and first- and second-strand cDNA synthesis. The carefully optimized reaction system has efficient reverse transcription activity and cDNA synthesis efficiency, and has good experimental compatibility.

Scope of application: This kit can be applied to the RNA fragmentation and double-stranded cDNA synthesis reaction of RNA library construction for the Illumina high-throughput sequencing platform.

Applicable sample input: 10 ng-1 μg total RNAs. As low as 500 pg mRNA of animals, plants and fungus.

Other recommended reagents

1. TIANSeq rRNA Depletion Kit (H/M/R) (4992363/4992364/4992391)
2. TIANSeq RNA Clean Beads (4992360/4992362/4992867)
3. TIANSeq Size Selection DNA Beads (4992358/4992359/4992979)

Features

Wide range of samples: This kit can be used to conduct fragmentation and double-strand cDNA synthesis for both mRNA and rRNA-removed RNA.

Efficient conversion: Optimized reaction system to ensure product stability and efficient double-strand cDNA synthetic efficiency.

Easy and simple operation: Integrated reaction process to simplify operation steps;

Good compatibility: Purified double-strand cDNA of the reaction product of the kit can be used directly in the construction of RNA-Seq library.

Precautions Please read the precautions before using this kit.

1. Please pay attention to avoiding cross-contamination between nucleic acid samples and products during operation.
2. Please use RNase-free tips and centrifuge tubes for the experiments.
3. Before the experiment, please clean the bench and treat it with RNase removal reagent, such as RNase Away (Molecular BioProducts, Inc), to ensure no RNase contamination.
4. Before the experiment, please read the instructions carefully. The samples can be stored at certain steps according to the instructions.
5. It is recommended to use high-quality RNA samples with RIN value ≥7.0 and good integrity to remove rRNA or isolate mRNA, otherwise, it will affect the RNA fragmentation and cDNA synthesis efficiency.

Protocol

I. RNA fragmentation and random primer combination

(I) Test preparations:

1. Take the rRNA-removed total RNAs or mRNA samples from the freezer at -80°C and thaw them slowly on ice.
2. Before the test, make sure the sample input of the rRNA-removed total RNA or mRNA is between 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.

(II) Procedure

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. Set up the reaction system according to the table below:

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

2. Selection of fragmentation conditions according to the table below. The temperature of the hot lid of PCR is set to be 105°C.

Insertion size (bp)	Reaction temperature	Reaction 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.

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:

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

Notes: If multiple sample reactions are performed simultaneously, it is recommended to prepare the mixture of 1st Strand Enzyme Mix and Nuclease-Free ddH_2O in advance in an appropriate centrifuge tube and then liquot into reaction tubes. It is suggested that the mixture should be prepared according to 1.1 times of the actual reaction number.

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:

Component	Volume (μl)
Synthesized first-strand cDNA	20
2nd Strand Buffer	8.5
2nd Strand Enzyme Mix	3.5
Nuclease-Free ddH_2O	48
Total	80

2. During the synthetic reaction of the second-strand cDNA in the PCR instrument, the temperature of the hot lid of PCR is set to be ≤40°C:

Step	Reaction temperature	Reaction 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. Purification of double-strand cDNA

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 magnetic beads at room temperature for 20 min.
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.