FastSelect rRNA Kit (Human)

N460

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

FastSelect rRNA Kit (Human) is designed to remove ribosomal RNA (rRNA; including cytoplasmic 28S, 18S, 5.8S, 5S rRNA, and mitochondrial 16S, 12S rRNA) from human total RNA, while retaining mRNA and other non-coding RNA. It is also applicable to both intact and partially degraded RNA samples (e.g., FFPE samples), and the ribosomal-depleted RNA can be used for analysis of mRNA and non-coding RNA (such as lncRNA). The kit is simple and fast to operate, and can quickly remove rRNA from human total RNA, thereby improving the efficiency of library preparation.

02/Components

	Components	N460-01 (24 rxns)	N460-02 (96 rxns)
■	Fast rRNA Removal Mix	24 μl	96 μl
□	Nuclease-free ddH_2O	1 ml	4 × 1 ml

03/Storage

Store at -30 ~ -15°C and transport at ≤0°C.

04/Applications

It is intended for human total RNA of 1 ng - 1 μg initial templates.

05/Self-prepared Materials

RNA quality control: Agilent RNA 6000 Pico Kit (Agilent #5067 - 1513).

RNA purification: VAHTS RNA Clean Beads (Vazyme #N412).

Other materials:

Agilent Technologies 2100 Bioanalyzer or other equivalent instruments, Nuclease-free PCR tube, PCR instrument, etc.

06/Notes

For research use only. Not for use in diagnostic procedures.

06-1/Kit Storage

1. The kit must be stored at -30 ~ -15°C. Please place on ice during use and store in time after use.
2. To avoid repeated freezing and thawing, it is recommended to store the remaining reagents in small aliquots after the first use.

06-2/RNA Sample Preparation

1. To ensure rRNA depletion efficiency, RNA samples should be free of salt ions (such as Mg^{2+} or guanidine salts) and organic compounds (such as phenol and ethanol).
2. To avoid DNA contamination, RNA samples can be treated with DNase I to remove DNA.
3. For RNA-seq samples, it is recommended that the initial amount of total RNA be higher than 100 ng to increase the library complexity.

06-3/Precautions for Operation

1. To avoid cross-contamination of samples, it is recommended to use filter tips and change the pipette tip after each sample.
2. Be sure to wear gloves for operation. After touching equipment outside the RNase-free space or other working areas, please change gloves.
3. Be sure to cap the tubes of all reagents immediately after use to avoid contamination.

07/Mechanism & Workflow

Take conventional RNA library preparation as an example

1. rRNA binds to rRNA antigens

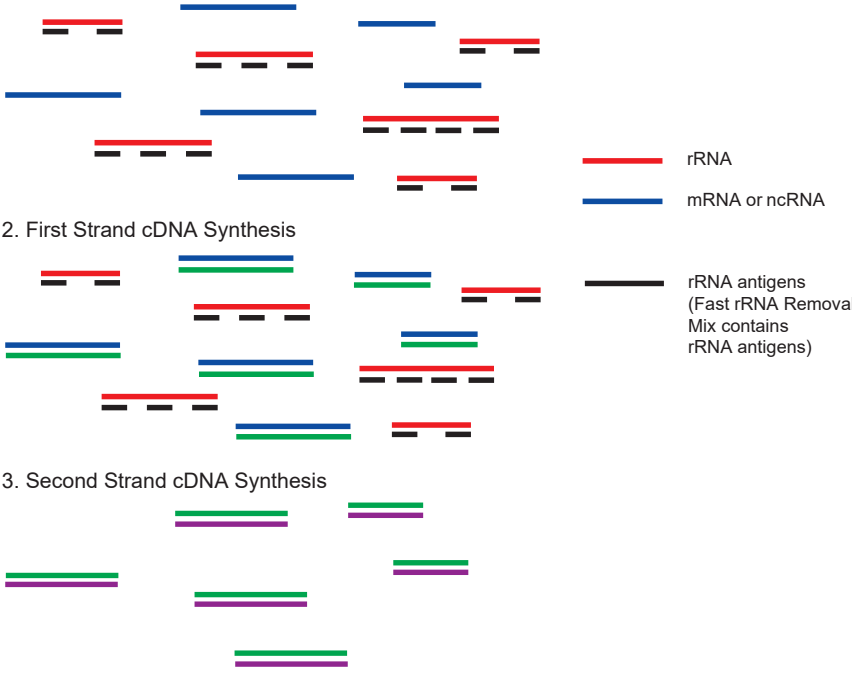

Fig 1. Mechanism of rRNA Removal

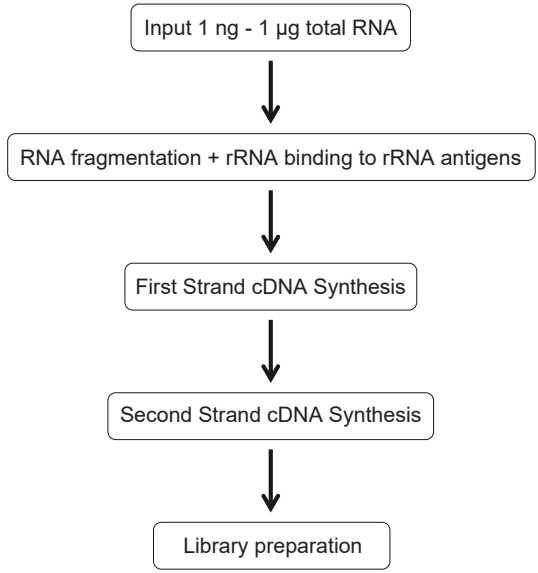

Fig 2. Simple Workflow of rRNA Depletion

08/Experiment Process

Protocol A: Use UltraClean Universal V8 RNA-seq Library Prep Kit for Illumina (Vazyme #UNR605) to perform library preparation as an example.

1. Prepare the total RNA sample: In a Nuclease-free centrifuge tube, dilute the total RNA to 7 μl with Nuclease-free ddH_2O and place on ice for later use.

2. Prepare the following reaction mix in a Nuclease-free PCR tube:

Components	Volume
Fast rRNA Removal Mix	1 μl
2 × Frag/Primer Buffer	8 μl
Total RNA	7 μl
Total	16 μl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

3. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
85°C*	6 min
75°C	1 min
55°C	1 min
4°C	Hold

*Set fragmentation programs according to the Insert size:

Insert size	Temperature	Time
150 - 200 bp	94°C	8 min
200 - 300 bp	94°C	5 min
250 - 400 bp	85°C	6 min
450 - 550 bp	85°C	5 min

4. Take out the components required for first strand cDNA synthesis from -30 ~ -15°C. Thaw on ice and mix by inverting, then centrifuge briefly to the bottom of the tube. Prepare the reaction system of first strand cDNA synthesis as follows:

Components	Volume
Reaction products from previous step	16 µl
1st Strand Buffer 3	7 µl
1st Strand Enzyme Mix 3	2 µl
Total	25 µl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

5. Perform the first strand cDNA synthesis reaction in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
25°C	10 min
42°C	15 min
70°C	15 min
4°C	Hold

6. Please refer to UNR605 instructions for second strand cDNA synthesis and subsequent experiments.

Protocol B: Use the UltraClean RNA Library Prep Kit for Illumina (Vazyme #UTR501 - UTR503) to perform library preparation as an example.

1. Prepare the total RNA sample: In a Nuclease-free centrifuge tube, dilute the total RNA to 9 µl with Nuclease-free ddH_2O and place on ice for later use.
2. Prepare the following reaction mix in a Nuclease-free PCR tube:

Components	Volume
Fast rRNA Removal Mix	1 µl
Total RNA	9 µl
Total	10 µl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

3. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
75°C	1 min
55°C	1 min
4°C	Hold

4. Thaw the components required for RNA reverse transcription at room temperature. Mix thoroughly by inverting for late use. Prepare the following mixture in a Nuclease-free PCR tube as follows:

Components	Volume
Reaction products from previous step	10 μl
10 × RT Mix	2 μl
HiScript III Enzyme Mix	2 μl
Oligo $(dT)_{20}VN$	1 μl
Random hexamers	1 μl
Nuclease-free ddH_2O	4 μl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

5. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
25°C	5 min
37°C	30 min
85°C	5 sec
4°C	Hold

6. Please refer to UTR501/UTR502/UTR503 instructions for RNA fragmentation and subsequent experiments.

Protocol C: Use UltraClean ds-cDNA Synthesis Module (+gDNA wiper) (Vazyme #UNR201) to perform cDNA synthesis as an example.

1. Prepare the gDNA digestion reaction system according to the following table:

Components	Volume
Total RNA	x μl
Reaction Buffer	2 μl
gDNA wiper	2 μl
Nuclease-free ddH_2O	To 17 μl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

2. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
37°C	15 min
85°C	7 min
4°C	Hold

3. Take out the components required for rRNA removal from -30 ~ -15°C. Thaw on ice and mix by inverting, then centrifuge briefly to the bottom of the tube. Prepare the reaction system of rRNA removal as follows:

Components	Volume
Reaction products from previous step	17 μl
Fast rRNA Removal Mix	1 μl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

4. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
75°C	1 min
55°C	1 min
4°C	Hold

5. Take out the components required for double-stranded cDNA Synthesis from -30 ~ -15°C. Thaw on ice and mix by inverting, then centrifuge briefly to the bottom of the tube. Prepare the reaction system of first strand cDNA synthesis as follows:

Components	Volume
Reaction products from previous step	18 μl
1st Strand Buffer 5	5 μl
1st Strand Enzyme Mix 3	2 μl

Mix thoroughly by gently pipetting up and down 10 times, then centrifuge briefly to the bottom of the tube.

6. Run the following program in the PCR instrument:

Temperature	Time
105°C (Heated lid)	On
25°C	5 min
42°C	10 min
85°C	5 min
4°C	Hold

7. Please refer to UNR201 instructions for second strand cDNA synthesis and subsequent experiments.

09/FAQ & Troubleshooting

◇ What is the recommended initial amount of RNA samples?

For samples used for RNA-Seq, the input amount of total RNA depends on the range of the RNA library kit used.

◇ If the library concentration is too low, why and how to improve it?

1. If the library yield is lower than other depletion methods, this is usually caused by the increased rRNA removal effect of this depletion method, which is normal. According to the test data, increasing the number of library amplification cycles by 1 - 2 is usually enough to meet the requirements of sequencing.

2. Using high-quality RNA samples as templates, the concentration of the library can meet the sequencing requirements. If you do not have high-quality RNA samples, you can try the following methods to improve it:

   ① Increase the input amount of initial templates, up to 1 μg.

   ② Add several replicate samples, purify and pool for amplification.

◇ Can FFPE samples or severely degraded samples be used to prepare libraries with this kit?

The following program is recommended for FFPE samples or heavily degraded RNA samples:

Temperature	Time
85°C*	6 min
75°C	2 min
70°C	2 min
65°C	2 min
60°C	2 min
55°C	2 min
37°C	2 min
25°C	2 min
4°C	Hold

* Set fragmentation programs according to the Insert size.

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