RNA Library Prep Kit (Premixed Version)

Product description

RNA Library Prep Kit (Premixed Version) is a kit specifically designed for transcriptome library construction on high-throughput sequencing platforms. It is suitable for high-quality eukaryotic total RNA, purified mRNA, and rRNA-depleted RNA samples, enabling efficient transcriptome library preparation. The kit provides two modules for second-strand synthesis, allowing for either non-stranded or strand-specific library construction as needed. Our product employs a premixed reagent format, which significantly simplifies the workflow and reduces pipetting steps, thereby greatly improving experimental efficiency.

Composition and storage conditions

Size / Components	K1810-24 rxns	K1810-96 rxns	Storage
Frag/Prime Buffer	480 µL	2× 960 µL	-20°C
1st strand Master Mix	216 µL	864 µL	-20°C
2nd strand Master Mix (with dNTP)	960 µL	4× 960 µL	-20°C
2nd strand Master Mix (with dUTP)	960 µL	4× 960 µL	-20°C
Ligation Master Mix	720 µL	4× 720 µL	-20°C
2×PCR Master Mix for library prep	600 µL	4× 600 µL	-20°C
Shipping: Dry Ice	**Shelf life:** 12 months		

Experimental operation

Experiment preparation

1) RNA Samples: If you use the mRNA Magnetic Purification Kit for RNA Library Prep (Cat. No. K1813), the required starting amount of total RNA is 10 ng–4 µg, and the total RNA must be intact (RIN ≥ 7). If you use an rRNA Depletion Kit, the required starting amount of total RNA is 10 ng–1 µg. If you use already purified mRNA or rRNA-depleted RNA directly for library preparation, the required starting amount is 0.5 ng–100 ng.

2) This kit does not provide Adaptors or the corresponding primers. Please prepare them according to your requirements.

3) Additional reagents and consumables required: PCR instrument, magnetic rack, DNA Clean Beads (Cat. No. K1811), 80% ethanol (freshly prepared), Nuclease-free ddH_2O, Low-retention nuclease-free PCR tubes and pipette tips, centrifuge tubes, etc.

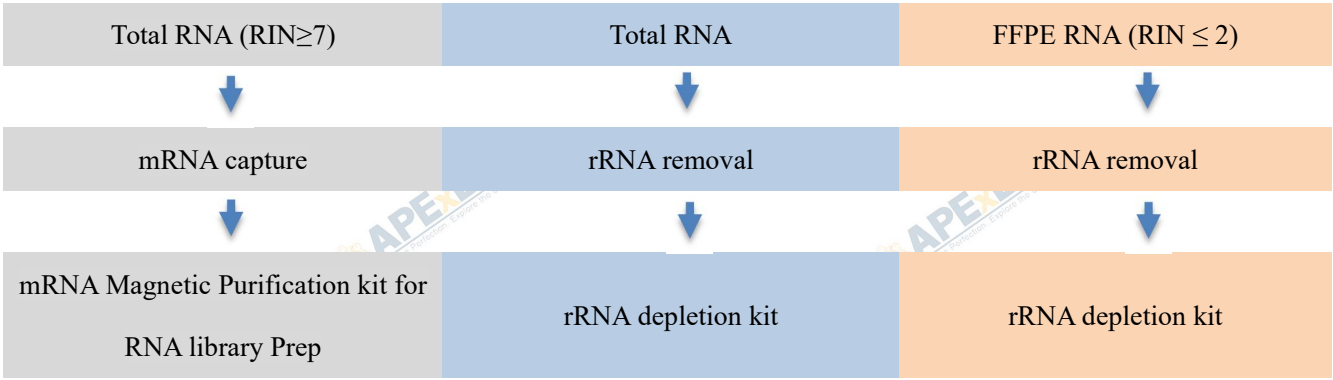

Fig.1 Different Input RNA samples is recommended to be handled with different methods

◆ Protocol

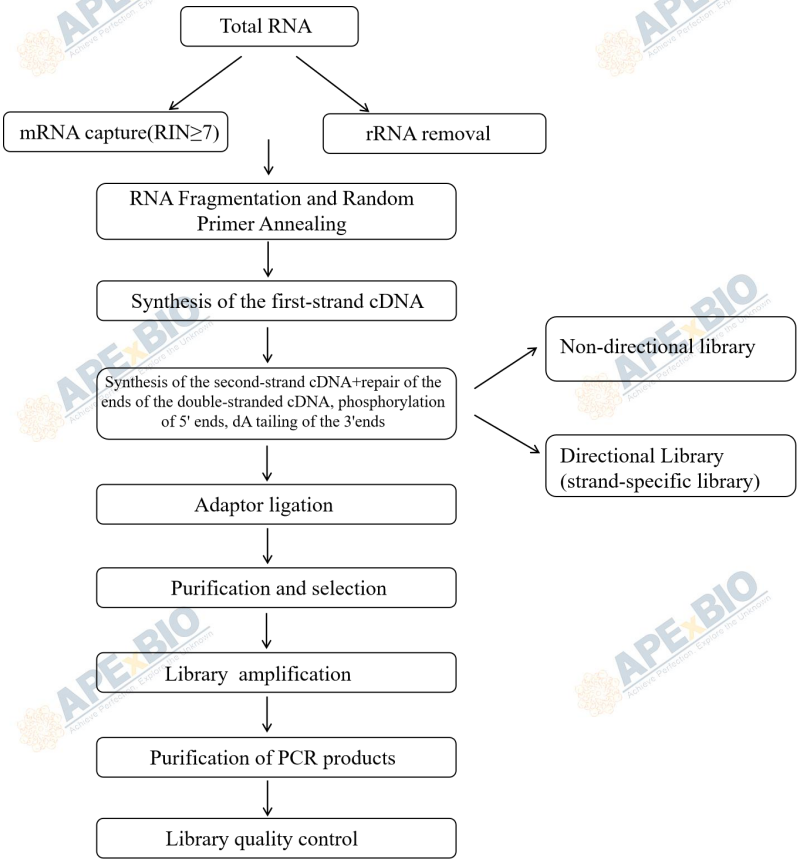

Fig.2 Schematic diagram of the overall operation process

1. Isolation and fragmentation of target RNA

As mentioned above, you can use our mRNA Magnetic Purification Kit for RNA Library Prep (Cat. No. K1813) to isolate mRNA from total RNA, or you may use an rRNA depletion kit. **The following protocol only describes the procedure using the mRNA Magnetic Purification Kit for RNA Library Prep**. If you are using an rRNA depletion kit, please refer

to the protocol provided with your kit, and finally use the Frag/Prime Buffer supplied with this kit for elution and fragmentation. For elution and fragmentation conditions, please refer to steps 1.1.16–1.1.18.

1.1 Section 1:

Using the mRNA Magnetic Purification Kit for RNA library prep (Cat. No. K1813) to enrich mRNA (steps 1.1.1-1.1.15 using reagents from K1813).

1.1.1 **Reagent Preparation**: Take the mRNA Magnetic Purification kit for RNA library Prep out of the 4°C refrigerator ahead and allow it to equilibrate to room temperature (about 30 min); Prepare some Nuclease-free ddH_2O.

1.1.2 **Sample preparation**: Dilute 10 ng-4 μg total RNA to 50 μL using Nuclease-free H_2O and place on ice for later use.

1.1.3 Invert or pipette to mix the mRNA Purification Beads thoroughly. Then take 50 μL beads and add it to the 50 μL total RNA sample. Gently pipette up and down 10 times until beads are homogenous.

1.1.4 Run the following program in the PCR instrument:

Temperature	Time
65°C	5 min
25°C	5 min
25°C	Hold

1.1.5 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min to separate the mRNA from the total RNA. After that, carefully remove and discard the supernatant.

1.1.6 Remove the sample from the magnetic rack, add 200 μL of Wash Buffer to resuspend the magnetic beads, and gently pipette 10 times to mix thoroughly.

1.1.7 Then place the sample on the magnetic rack and incubate at room temperature for about 5 min. After the solution is clear, carefully remove the supernatant.

1.1.8 Repeat steps1.1.6-1.1.7 once.

1.1.9 Remove the tube from the magnetic rack, add 50 μL of Tris Buffer, and gently pipette up and down 10 times.

1.1.10 Run the following program in the PCR instrument:

Temperature	Time
80°C	2 min
25°C	+∞

1.1.11 Take the sample out of the PCR instrument, add 50 μL of mRNA Binding Buffer (2×), and gently pipette up and

down 10 times.

1.1.12 Incubate at room temperature for 5 min to allow the mRNA to bind to the magnetic beads.

1.1.13 Place the sample on the magnetic rack and incubate at room temperature for 5 min. After the solution becomes clear, carefully remove and discard the supernatant.

1.1.14 Remove the sample from the magnetic rack, add 200 µL of Wash Buffer to resuspend the magnetic beads, and gently pipette up and down 10 times.

1.1.15 Place the sample on the magnetic rack and incubate at room temperature for 5 min. After the solution becomes clear, remove and discard all the supernatant.

***Note:** You can first use a 200 µL pipette to remove the liquid, then switch to a 10 µL pipette to remove the remaining liquid. If there is too much residual liquid in this step, it may affect the fragmentation effect.

1.1.16 Remove the sample from the magnetic rack, add 18 µL of Frag/Prime Buffer to resuspend the magnetic beads, and gently pipette up and down 10 times.

1.1.17 Place the sample in a PCR instrument for elution and fragmentation:

Temperature	Time
85°C	6 min
4°C	+∞

***Note:** Please select the appropriate temperature and time according to the size of the product you need.

Platform	Illumina		
The condition of fragmentation	94°C 5 min	85°C 6 min	85°C 6 min
Inserted fragment length (bp)	180 - 280	280 - 380	380 - 480
Library length (bp)	300 - 400	400 - 500	500 - 600

1.1.18 After RNA fragmentation is complete, to prevent poly(A) tail RNA from binding to the magnetic beads, please immediately place the sample on a magnetic rack. Once the solution becomes clear, promptly transfer 16 µL of the supernatant to a new nuclease-free PCR tube, and immediately proceed to the first-strand cDNA synthesis step. Be careful not to aspirate any magnetic beads.

***Note:** The components required for the synthesis of double-stranded cDNA can be taken out of the -20°C refrigerator for thawing in advance.

1.2 Section 2:

Library construction starting from purified mRNA, rRNA-depleted RNA, or small amounts of total RNA.

This protocol should be used with the 2× Frag/Prime Buffer for RNA Library Prep (Cat. No. K1814). It is suitable for transcriptome library construction starting from 0.5–100 ng of purified mRNA, rRNA-depleted RNA, or total RNA.

1.2.1 Prepare the reaction system according to the following table:

Reagent	Volume
2 × Frag/Prime Buffer for RNA library Prep	8 μL
RNA	8 μL
Total	16 μL

1.2.2 Invert, or gently pipette to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube. Then place the sample in a PCR instrument for fragmentation:

Temperature	Time
85°C	6 min
4°C	+∞

***Note:** Please select the appropriate temperature and time according to the size of the product you need.

Platform	Illumina		
The condition of fragmentation	94°C 5 min	85°C 6 min	85°C 6 min
Inserted fragment length (bp)	180 - 280	280 - 380	380 - 480
Library length (bp)	300 - 400	400 - 500	500 - 600

1.2.3 From the fragmentation step to the first-strand cDNA synthesis, the process should not be paused, as mRNA is prone to degradation under these conditions. The components required for the double-stranded cDNA synthesis step can be taken out from -20°C in advance and thawed on ice for later use.

2. Double-stranded cDNA synthesis

2.1 Synthesis of the first-strand cDNA

2.1.1 Invert and mix the thawed 1st strand Master Mix, briefly centrifuge, and prepare the reaction system for the synthesis of the first strand cDNA as shown in the following table:

Reagent	Volume
Fragmented mRNA from previous step	16 μL
1st strand Master Mix	9 μL

Total	25 μL

2.1.2 Thoroughly mix and briefly centrifuge to collect the liquid to the bottom of the tube.

2.1.3 Place the sample in a PCR instrument for the first-strand cDNA synthesis reaction:

Temperature	Time
105°C (Heated lid)	On
25°C	10 min
50°C	15 min
80°C	10 min
4°C	Hold

2.1.4 After the reaction is complete, immediately proceed to the second-strand cDNA synthesis reaction. The components required for step 2.2 can be taken out from -20°C in advance and placed on ice for later use.

2.2 Synthesis of the second-strand cDNA

2.2.1 Prepare the reaction system for the synthesis of the second-strand cDNA as shown in the following table:

Reagent	Volume
1st Strand cDNA from previous step	25 μL
2nd strand Master Mix (with dNTP or dUTP)	40 μL
Total	65 μL

***Note:** When performing non-stranded RNA-seq, use the 2nd strand Master Mix (with dNTP); when performing strand-specific RNA-seq, use the 2nd strand Master Mix (with dUTP).

2.2.2 Invert, or gently pipette to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

2.2.3 Place the sample in a PCR instrument for the second-strand cDNA (ds cDNA) synthesis reaction:

Temperature	Time
105°C (Heated lid)	On
16°C	30 min
72°C	15 min
4°C	Hold

2.2.4 The components required for Adapter ligation can be taken out from -20°C in advance and placed on ice for later use.

3. Adapter ligation

3.1 Prepare the reaction system for Adapter ligation as shown in the following table:

Reagent	Volume
ds cDNA from previous step	65 µL
Adapter	5 µL
Ligation Master Mix	30 µL
Total	100 µL

***Note:**

a. It is recommended to add the Adapter to the ds cDNA from the previous step first, mix thoroughly, and then add the Ligation Master Mix.

b. The volume of Adapter to be added is fixed at 5 µL. Please dilute the Adapter to the appropriate concentration according to the initial amount of RNA input. Use 0.1× TE buffer as the diluent for the Adapter.

Section 1: Input Total RNA	Section 2: Purified mRNA or rRNA-depleted RNA	Illumina® Adapter concentration
≥1 µg	100 ng	5 µM
500 ng	10 ng	3 µM
100 ng	1 ng	1.5 µM
10 ng	0.5 ng	1 µM

c. The Ligation Master Mix contains a high concentration of PEG and is very viscous. Please pipette slowly to avoid sampling errors.

d. There are various types of adaptors available on the market. Please choose according to your experimental requirements.

3.2 Invert, or gently pipette to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

3.3 Place the sample in a PCR instrument for Adapter ligation reaction:

Temperature	Time
105°C (Heated lid)	OFF
20°C	15 min
4°C	Hold

***Note:** The Adapter ligation products can be temporarily stored at 2-8°C for 1 hour.

4. Purification or Selection of the Adapter ligated products

This step provides two options; please choose according to your needs:

- **Purification Option**: This is a single-round purification without any size-selection. This option could yield a wide-range library of fragments >200 bp and removes adapter residues.

- **Seletion Option:** This involves purification plus two rounds of size-selection. The selection scheme can be adjusted according to your experimental objectives.

The following steps require DNA Clean Beads (Cat. No. K1811). Please take the DNA Clean Beads from the 4°C refrigerator in advance and allow them to equilibrate to room temperature (about 30 min) before use.

4.1 Purification Option

4.1.1 Invert or pipette to mix the DNA Clean Beads thoroughly. Then take 60 μL (0.6×) beads and add it to the DNA sample. Gently pipette up and down 10 times until beads are homogenous.

4.1.2 Incubate for 10 min at room temperature to allow DNA to bind to the beads.

4.1.3 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min, carefully remove and discard the supernatant.

4.1.4 Keeping the sample on the magnetic rack, add 200 μL of freshly prepared 80% ethanol solution to rinse the magnetic beads, incubate for 30 sec at room temperature, and carefully remove the supernatant.

> ***Note:** Please rinse the magnetic beads with freshly prepared 80% ethanol. Using 80% ethanol with long time storage may result in an increase of residual impurities and a reduction in library yield.

4.1.5 Repeat step 4.1.4 once for a total of two times of rinse. When removing the supernatant at last, a 10 μL pipette should be used to aspirate the remaining liquid completely.

4.1.6 Keep the sample on the magnetic rack and dry the beads with the lid open for approximately 5-10 min at room temperature.

> ***Note:** When drying magnetic beads, please avoid reducing the recovery efficiency due to over-drying.

4.1.7 Remove the sample from the magnetic rack, add 22 μL of Nuclease-free ddH_2O, gently pipette to mix thoroughly, incubate at room temperature for 2 min, then place it on the magnetic rack. After the solution becomes clear (about 5 min), carefully transfer 20 μL of supernatant into a new Nuclease-free PCR tube. Then proceed to the PCR amplification step (step 5.1).

4.2 Selection Option

This option involves purification step plus two rounds of size-selection. The selection scheme can be adjusted according to your experimental objectives.

I First, purify the Adapter ligated products using 0.6× magnetic beads

4.2.1 Invert or pipette to mix the DNA Clean Beads thoroughly. Then take 60 μL (0.6×) beads and add it to the products. Gently pipette up and down 10 times until beads are homogenous.

4.2.2 Incubate for 10 min at room temperature to allow DNA to bind to the beads.

4.2.3 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min, carefully remove and discard the supernatant.

4.2.4 Keeping the sample on the magnetic rack, add 200 μL of freshly prepared 80% ethanol solution to rinse the magnetic beads, incubate for 30 sec at room temperature, and carefully remove the supernatant.

***Note:** Please rinse the magnetic beads with freshly prepared 80% ethanol. Using 80% ethanol with long time storage may result in an increase of residual impurities and a reduction in library yield.

4.2.5 Repeat step 4.2.4 once for a total of two times of rinse. When removing the supernatant at last, a 10 μL pipette should be used to aspirate the remaining liquid completely.

4.2.6 Keep the sample on the magnetic rack and dry the beads with the lid open for approximately 5-10 min at room temperature.

***Note**: When drying magnetic beads, please avoid reducing the recovery efficiency due to over-drying.

4.2.7 Remove the sample from the magnetic rack, add 102 μL of Nuclease-free ddH_2O, gently pipette to mix thoroughly, incubate at room temperature for 2 min, then place it on the magnetic rack. After the solution becomes clear (about 5 min), carefully transfer 100 μL of supernatant into a new Nuclease-free PCR tube.

II Second, two rounds of size-selection with DNA Clean Beads

The following is an illustration with a fragmentation condition at 85°C for 6 minutes, and an insert size of 280–380 bp. For libraries of other fragment lengths, please refer to the table below to select the appropriate volume of magnetic beads.

Platform		Illumina		
Library length (bp)		300-400	400-500	500-600
The condition of fragmentation		94℃ 5 min	85℃ 6 min	85℃ 6 min
Inserted fragment length (bp)		180 - 280	280 - 380	380 - 480
Full Length Adapter	Volume of beads for 1st round (μL)	65(0.65×)	60(0.6×)	50(0.5×)
	Volume of beads for 2nd round (μL)	10(0.1×)	10(0.1×)	10(0.1×)
Inserted fragment length (bp)		180 - 280	280 - 380	380 - 480
Stubby Adapter	Volume of beads for 1st round (μL)	65(0.65×)	60(0.6×)	50(0.5×)
	Volume of beads for 2nd round (μL)	10(0.1×)	10(0.1×)	10(0.1×)

***Note:** The library size mentioned here refers to the final library length. Variations in the volume of beads added during size selection will affect the final library size. Note that the bead volume ratio used during size selection is the ratio to the initial DNA volume. For example, if the initial volume is 100 μL of DNA solution, the first round of size selection uses 60 μL of beads, which is 60% of the initial 100 μL, and is hence noted as 0.6×. The second round uses 10 μL of beads, which is still 10% of the initial 100 μL DNA solution, i.e., 0.1×, rather than 10% of the 150 μL supernatant that is transfered.

4.2.8 Invert or pipette to mix the DNA Clean Beads thoroughly. Then take 60 μL (0.6×) beads and add it to the products. Gently pipette up and down 10 times until beads are homogenous.

4.2.9 Incubate for 10 min at room temperature to allow DNA to bind to the beads.

4.2.10 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min. After the solution becomes clear, carefully transfer 150 μL of supernatant into a new Nuclease-free PCR tube. **Note that the supernatant**

should be retained in this step!

4.2.11 Add 10 μL (0.1×) of magnetic beads to the supernatant from step 4.2.10 and gently pipette 10 times to mix thoroughly.

4.2.12 Incubate for 10 min at room temperature to allow DNA to bind to the beads.

4.2.13 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min, carefully remove and discard the supernatant.

4.2.14 Keeping the sample on the magnetic rack, add 200 μL of freshly prepared 80% ethanol solution to rinse the magnetic beads, incubate for 30 sec at room temperature, and carefully remove the supernatant.

4.2.15 Repeat step 4.2.14 once for a total of two times of rinse. When removing the supernatant at last, a 10 μL pipette should be used to aspirate the remaining liquid completely.

4.2.16 Keep the sample on the magnetic rack and dry the beads with the lid open for approximately 5-10 min at room temperature.

*__Note:__ When drying magnetic beads, please avoid reducing the recovery efficiency due to over-drying.

4.2.17 Remove the sample from the magnetic rack, add 22 μL of Nuclease-free ddH2O, gently pipette to mix thoroughly, incubate at room temperature for 2 min, then place it on the magnetic rack. After the solution becomes clear (about 5 min), carefully transfer 20 μL of supernatant into a new Nuclease-free PCR tube. Proceed to the PCR amplification step (step 5.1).

*__Note:__

a. When transferring the supernatant, be careful not to disturb the magnetic beads, as even a very small amount of residual beads can affect the subsequent library yield.

b. The purified product can be temporarily stored at -30 to -15°C for up to 24 hours.

5. Library amplification

5.1 Prepare the PCR reaction system according to the adaptor used:

Reagent	Volume
Purified adapter ligated products from previous step	20 μL
2× PCR Master Mix for library prep	25 μL
PCR Primer Mix	5 μL
Total	50 μL

*__Note:__ Please use the Primer that corresponding to the adaptor you actually used.

5.2 Invert, or gently pipette to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

5.3 Place the sample in a PCR instrument for Library amplification reaction:

Procedure	Temperature	Time	Cycles
Heated lid	105°C	On	
Initial Denaturation	98°C	30 s	1
Denaturation	98°C	10 s	10-18
Annealing	60°C	30 s	
Extension	72°C	30 s	
Complete extension	72°C	1 min	1
	4°C	Hold	

***Note:**

a. The proportion of mRNA in an equal amount of total RNA may vary depending on the source species or individual differences. According to the actual situation of the samples, the number of PCR cycles can be appropriately adjusted, generally ranging from 10 to 18 cycles. You can also adjust the number of amplification cycles based on experimental results and yield.

b. Recommended number of cycles:

		Cycles	
Section 1: Total initial input of RNA	Section 2: Purified mRNA or rRNA-depleted RNA	Non-stranded RNA-seq	Strand-specific RNA-seq
＞1μg		10	11
1μg	100 ng	11	12
500 ng	10 ng	13	14
100 ng	1 ng	15	16
10 ng	0.5 ng	17	18

5.4 Purification of PCR products

5.4.1 Invert or pipette to mix the DNA Clean Beads (Cat. No. K1811) thoroughly. Then take 45 μL (0.9×) beads and add it to the DNA sample. Gently pipette up and down 10 times until beads are homogenous.

***Note:** Please take the DNA Clean Beads out of the 4℃ refrigerator in advance and equilibrate to room temperature (about 30 minutes).

5.4.2 Incubate for 10 min at room temperature to allow DNA to bind to the beads.

5.4.3 Then place the sample on a magnetic rack and incubate at room temperature for about 5 min, carefully remove and discard the supernatant.

5.4.4 Keeping the sample on the magnetic rack, add 200 μL of freshly prepared 80% ethanol solution to rinse the magnetic beads, incubate for 30 sec at room temperature, and carefully remove the supernatant.

5.4.5 Repeat step 5.4.4 once for a total of two times of rinse. When removing the supernatant at last, a 10 μL pipette should be used to aspirate the remaining liquid completely.

5.4.6 Keep the sample on the magnetic rack and dry the beads with the lid open for approximately 5-10 min at room

temperature.

*Note: When drying magnetic beads, please avoid reducing the recovery efficiency due to over-drying.

5.4.7 Remove the sample from the magnetic rack, add 22 μL of Nuclease-free ddH_2O, gently pipette to mix thoroughly, incubate at room temperature for 2 min, then place it on the magnetic rack. After the solution becomes clear (about 5 min), carefully transfer 20 μL of supernatant into a new Nuclease-free PCR tube.

*Note: Be careful not to disturb the beads when removing the supernatant at the end, to avoid affecting subsequent steps.

6. Library quality control

Under normal circumstances, the quality of the constructed library can be evaluated by assessing its size distribution and concentration.

Take 1 μL of the purified PCR product for capillary electrophoresis analysis. If the library is high quality, a narrow peak should appear at the expected size. If a peak appears at 90–140 bp, it indicates the presence of adapter dimer contamination in the library. In this case, dilute the library with nuclease-free ddH_2O to 50 μL, and then perform PCR product purification again.

For concentration detection, methods based on double-stranded DNA fluorescent dyes, such as Qubit® or PicoGreen®, or absolute quantification qPCR can be used. Concentration detection of the library should not use spectrophotometric methods, such as NanoDrop®.

Notes

1 Before use, thaw all components of the kit on ice. Mix thoroughly by inverting several times, briefly centrifuge, and keep on ice until use.

2 Gently pipette to mix solutions containing RNA samples. Avoid vortexing to prevent RNA fragmentation and ensure that library fragment size is not affected.

3 mRNA enriched by the Poly(A) method or RNA after rRNA depletion should be processed to the next step as soon as possible to avoid RNA degradation.

4 After removing magnetic beads from 2–8°C storage, allow them to equilibrate to room temperature; otherwise, the capture efficiency may decrease. Always mix the beads thoroughly before each use.

5 Wash the magnetic beads with freshly prepared 80% ethanol to avoid residual impurities and reduced library yield. During washing, always keep the PCR tube on the magnetic rack and avoid disturbing the beads.

6 Materials used before second-strand synthesis must be nuclease-free. For second-strand synthesis and subsequent steps,

use DNase-free materials.

7 After thoroughly mixing the sample with magnetic beads and placing them on the magnetic rack for separation, wait until the solution is completely clear before aspirating the supernatant, leaving behind 2–3 μL. If beads are accidentally aspirated, it may reduce yield, affect separation efficiency and subsequent enzymatic reactions. In this case, remix the beads and liquid again on the magnetic rack. Due to differences in magnetic strength, the default separation time may need to be extended or shortened to ensure complete separation of beads and liquid.

8 During the second 80% ethanol wash of the magnetic beads, aspirate the supernatant as thoroughly as possible to minimize residual impurities and reduce room temperature drying time.

9 Before eluting the product, allow the beads to air dry at room temperature. Insufficient drying may result in ethanol residue affecting subsequent reactions, while over-drying may cause bead cracking and reduce purification yield. Typically, air drying at room temperature for 5–10 minutes is sufficient. Do not dry by heating, such as in a 37°C oven.

10 It is recommended to use pipette tips with filters.

11 This product is for research use only!

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