VAHTS™ mRNA-seq V2 Library Prep Kit for Illumina®

NR601

Version 8.1

Vazyme biotech co., ltd.

Introduction

The Vazyme VAHTS mRNA-seq V2 Library Prep Kit for Illumina® is specially designed for the preparation of ready-to-use transcriptome libraries for next generation sequencing (NGS) platforms of Illumina. Starting from 0.1 μg - 4 μg total RNA of animal, plant, orfungal, this kit can be used for mRNA isolation, fragmentation, synthesis of cDNA, end-repair, dA-tailing, adapter-ligation, size selection of library, and library amplification. The kit has been tested and promised to provide stable and repeatable output cDNA library.

Components of kits

	Component	NR601-01 (24 rxn)	NR601-02 (96 rxn)
NR 1	mRNA Capture Beads	1.2 ml	4.8 ml
	Beads Binding Buffer	1.2 ml	4.8 ml
	Beads Wash Buffer	9.6 ml	38.4 ml
	Tris Buffer	1.2 ml	4.8 ml
NR 2	Frag/Prime Buffer	468 μl	2×936 μl
	1st Strand Buffer	144 μl	576 μl
	1st Strand Enzyme Mix	48 μl	192 μl
	2nd Strand Buffer	480 μl	2×960 μl
	2nd Strand Enzyme Mix	120 μl	480 μl
NR 3	End Prep Mix	960 μl	4×960 μl
	dA-Tailing Bufer Mix	240 μl	960 μl
	dA-Tailing Enzyme Mix	60 μl	240 μl
	Ligation Mix	60 μl	240 μl
	Stop Ligation Mix	120 μl	480 μl
	PCR Primer Mix	120 μl	480 μl
	Amplification Mix1	600 μl	4×600 μl

Storage

NR1:Storeat 2 - 8˚C (**DO NOT** store at -20˚C)
NR2:Storeat -20˚C
NR3:Storeat -20˚C

Applications

Requirements for Starting Materials: 0.1 μg - 4 μg animal, plant, or fungal total RNA with high quality. It is recommended to use an Agilent 2100 Bioanalyzer to analyze the total RNA. The RIN (RNA integrity number) value should be ≥ 7.0. Using degraded total RNA for library construction will lead to in 3'bias in RNA-seq. The ratio of OD260/OD280 should be between 1.8 and 2.1.

Information of Transcripts: This kit is applicable to mRNA related analysis with RNA-seq, including gene expression analysis, single nucleotide variation calling, alternative splicing / fusion detection, and target transcriptome analysis.

If stranded transcriptome library is under consideration, please use the VAHTS Stranded mRNA-seq Library Prep Kit for Illumina® (Vazyme,#NR602) for library construction.

If non-coding RNA (i.e.lnc-RNA) is under consideration, please use the VAHTS Total RNA-seq (H/M/R) Library Prep Kit for Illumina® (Vazyme,#NR603) for library construction.

Additional Materials Required

DNA Clea nBeads: VAHTS DNA Clean Beads (Vazyme,#N411) or Agencourt® AMPure® XPBeads (Beckman Coulter, #A63880, #A63881, #A63882).

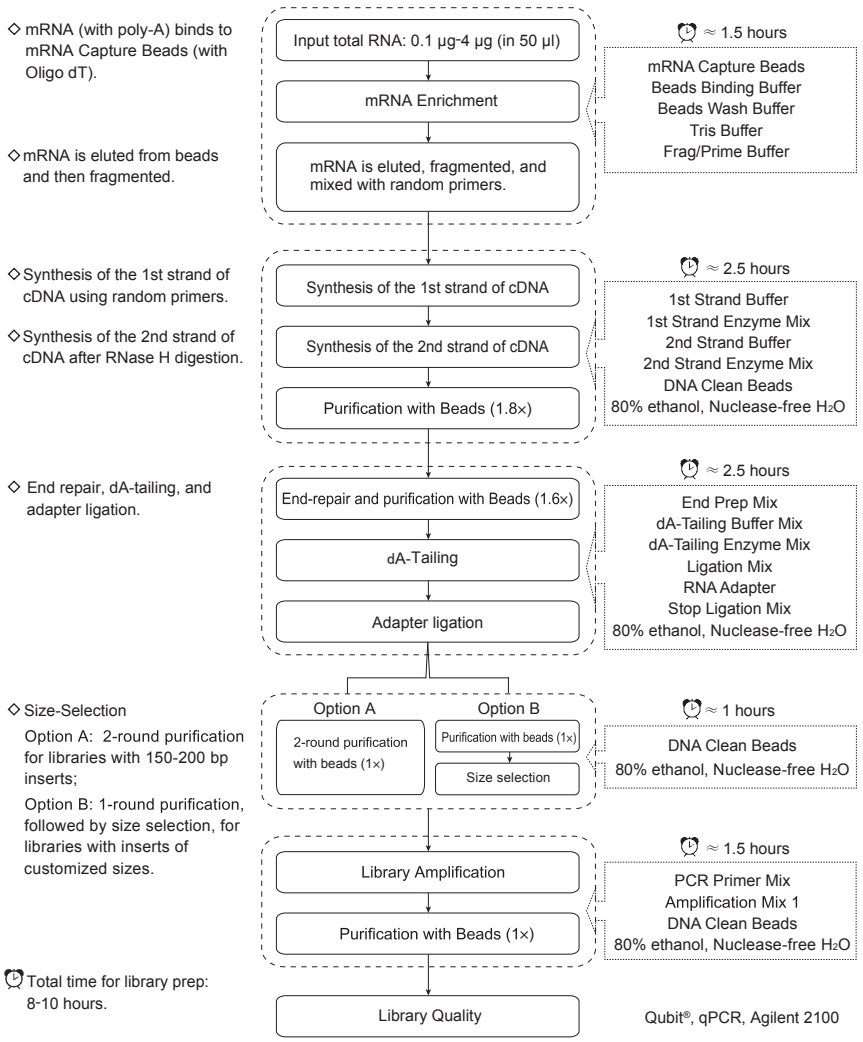
RNA Analysis: Agilent RNA 6000 Pico Kit (Agilent, #5067-1513).

Library Analysis: Agilent DNA 1000 Kit (Agilent, #5067-1504).

Adapters: VAHTS RNA Adapters Set 1 - Set 2 for Illumina® (Vazyme, #N803, #N804), or VAHTS RNA Adapters Set 3 - Set 6 for Illumina® (Vazyme, #N809, #N810, #N811, #N812).

Other Materials: Fresh Ethanol (80%), Nuclease-free Water, Nuclease-free PCR tubes, Low absorption EP tubes, Agilent 2100 Bioanalyzer, Thermocyler (PCRinstrument) ,Magnetic stand.

Mechanism & Workflow



Protocol

1. mRNA Purification and Fragmentation

1.1. Equilibrate NR1 (mRNA Capture Beads, Beads Wash Buffer, Tris Buffer, and Beads Binding Buffer) to room temperature.

Note: DO NOT vortex the mRNA Capture Beads, Beads Wash Buffer, and Beads Binding Buffer!

1.2. Prepare the RNA sample carefully b ydissolving 0.1 μg - 4 μg of total RNA in 50 μl of Nuclease-free Water in a Nuclease-free PCR tube. Keep the RNA solution on ice and proceed to the next step as soon as possible.

Note: DO NOT vortex the RNA solution!

1.3. Suspend the mRNA Capture Beads thoroughly by inverting or vortexing, and pipet 50 µl of beads into 50 µl of the dissolved total RNA. Mix thoroughly by pipetting up and down for 10 times.

1.4. Incubate the sample in a thermostatic device (i.e. a PCR machine) at 65℃ for 5 min to denature the RNA, then incubate at 25℃ for 5 min to make the mRNA bind to the mRNA Capture Beads.

1.5. Put the sample onto a magnetic stand. Wait until the soultion clarifies (about 5 min), then carefully discard the supernatant without disturbing the beads.

1.6. Take the sample out of the magnetic stand. Add 200 µl of Beads Wash Buffer, and mix thoroughly by pipetting up and down for 10 times. Put the sample back to the magnetic stand. Wait until the soultion clarifies (about 5 min),then carefully discard the supernatant without disturbing the beads.

1.7. Take the sample out of the magnetic stand, and add 50 µl of Tris Buffer to re-suspend the beads thoroughly by pipetting up and down for 10 times.

1.8. Incubate the sample in a thermostatic device (PCR machine) at 80℃ for 2 min and then hold at 25℃ to release mRNA.

1.9. Add 50 µl of Beads Binding Buffer, mix thoroughly by pipetting up and down for 10 times.

1.10. Incubate at room temperature for 5 min to make the mRNA bind to the beads.

1.11. Place the sample on the magnetic stand to isolate the mRNA from total RNA. Wait until the soultion clarifies (about 5 min), then carefully discard the supernatant without disturbing the mRNA Capture Beads.

1.12. Take the sample out of the magnetic stand, add 200 µl of Beads Wash Buffer, and mix thoroughly by pipetting up and down for 10 times. Place the tube on the magnetic separation rack. Wait until the soultion clarifies (about 5 min),then carefully discard the supernatant without disturbing the mRNA Capture Beads.

Note: It is highly recommended to use a 10-µl pipettor to remove the residual supernatant in this step.

1.13. Take the sample out of the magnetic stand, add 19.5 µl of Frag/Prime Buffer to re-suspend the beads thoroughly by pipetting up and down for 10 times. Incubate the sample in a PCR device and set programs according to the fragment size required:

- For **150-200 bp insert**: incubate at 94℃ for 8 min,then hold at 4℃.
- For **200-300 bp insert**: incubate at 94℃ for 5 min,then hold at 4℃.
- For **250-450 bp insert**: incubate at 85℃ for 6 min,then hold at 4℃.
- For **450-550 bp insert**: incubate at 85℃ for 5 min,then hold at 4℃.

1.14. Place the sample on he tmagnetic stand. Wait until the soultion clarifies (about 5 min), and pipet 17 µl of supernatant into a new Nuclease-free PCR tube,then immediately proceed to **Step 2.Synthesis of Double Strand cDNA**.

2. Synthesis of Double Strand cDNA

2.1. Thaw the 1st Strand buffer and mix it thoroughly by inverting the tube. Prepare the reaction solution to synthesize the first strand cDNA as follows:

Fragmented mRNA	17 µl	
1st Strand Buffer	6 µl	
1st Strand Enzyme Mix	2 µl	
Total	25 µl	

Mix thoroughly by gently pipetting up and down for 10 times.

2.2. Put the sample in a PCR instrument and run the following program (Hot Lid Temperature: 105℃) :

25 ℃	10 min
42 ℃	15 min
70 ℃	15 min
4 ℃	Hold

Immediately proceed to **Step2.3** for the 2nd Strand Synthesis of cDNA.

2.3. Thaw the 2nd Strand Buffer and mix it thoroughly by inverting the tube. Prepare the following reaction solution to synthesize the 2nd strand cDNA:

1st Strand cDNA	25 µl
2nd Strand Buffer	20 µl
2nd Strand Enzyme Mix	5 µl
Total	50 µl

Mix thoroughly by gently pipetting up and down for 10 times.

2.4. Put the sample in a PCR instrument and run the following program (Hot Lid Temperature: 30˚C) :

16 ˚C	60 min
4 ˚C	Hold

Note: The double strand cDNA products can be stored at 4˚C for less than 60 min.

2.5. Purification of double strand cDNA

a. Equilibrate the VAHTS DNA Clean Beads to room temperature.

b. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Pipet 90 ul (1.8x)of beads into the above sample. Mix thoroughly by pipetting up and down for 10 times.

c. Incubate at room temperature for 10 min.

d. Place the sample on a magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on magnetic stand and carefully discard the supernatant without disturbing the beads.

e. Keeping the sample on the magnetic stand, add 200 ul of ethanol to rinse the beads. **DO NOT re-suspend the beads!** Incubate at room temperature for 30 sec and carefully discard the supernatant without disturbing the beads.

f. Repeat the Step e.

g. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5-10 min.

h. Take the sample out of the magnetic stand. Add 62.5 ul of Nuclease-free water to elute the cDNA. Mix thoroughly by vortexing or pipetting and incubate for 2 min at room temperature. Put the tube on the magnetic stand and wait until the solution clarifies (about 5 min). Carefully transfer 60 ul of supernatant to a new Nuclease-free PCR tube without disturbing the beads.

Note: The dilution can be stored at -20˚C for 24 hours.

3. End Repair

3.1. Thaw the End Prep Mix and mix it thoroughly by inverting the tube. Prepare the reaction solution as follows:

ds cDNA	60 µl
End Prep Mix	40 µl
Total	100 µl

Mix thoroughly by gently pipetting up and down for 10 times.

3.2. Put the sample in a PCR instrument and run the following program for end- repair (Hot Lid Temperature: 105˚C) :

30˚C	30 min
4˚C	Hold

Note: the end-repair product can be stored at 4˚C for less than 60 min.

3.3. Purification of the end-repair products:

a. Equilibrate the VAHTS DNA Clean Beads to room temperature

b. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Pipet 160 ul (1.6x) of beads into the above sample. Mix thoroughly by pipetting up and down for 10 times.

c. Incubate at room temperature for 10 min

d. Place the sample on a magnetic stand. Wait until the solution clarifies (about 5 min). Keep the sample on the magnetic stand and carefully discard the supernatant without disturbing the beads.

e. Keeping the sample on the magnetic stand, add 200 ul of freshly prepared 80% ethanol to rinse the beads. **DO NOT re-suspend the beads!** Incubate at room temperature for 30 sec and carefully discard the supernatant without disturbing the beads.

f. Repeat the Step e.

g. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 10 min.

h. Take the sample out of the magnetic stand. Add 20 of Nuclease-free Water to elute DNA. Mix thoroughly by vortexing or pipetting and incubate at room temperature for 2 min. Place the tube on the magnetic stand and wait until solution clarifies (about 5 min). Carefully transfer 17.5 ul of supernatant to a new Nuclease-free PCR tube.

Note: The dilution can be stored at -20˚C for 24 hours.

4. dA-Tailing

4.1. Thaw the dA-tailing Buffer Mix and mix it thoroughly by inverting the tube. Prepare the reaction solution as follows:

Purified End-Repair Products	17.5 μl	
dA-tailing Buffer Mix	10 μl	■
dA-tailing Enzyme Mix	2.5 μl	■
Total	30 μl	

4.2. Put the sample in a PCR instrument and run the following program for dA-Tailing (Hot Lid Temperature 105˚C):

37˚C	30 min
70˚C	5 min
4˚C	Hold

Immediately proceed to **Step 5. Adapter Ligation** .

Note: The dA-tailing products can be stored at 4˚C for less than 60 min.

5.Adapter Ligation

5.1. Thaw the RNA Adapter and mix it thoroughly by inverting the tube. Prepare the reaction solution as follows:

Purified dA-ailing Products	30 μl	
Ligation Mix	2.5 μl	■
RNA Adapter*	2.5 μl	□
Total	35 μl	

*VAHTS RNA Adapters Set 1 for Illumina® (Vazyme, #N803) contains 12 adapters (Adapter 1 to 12).

VAHTS RNA Adapters Set 2 for Illumina® (Vazyme, #N804) contains 12 adapters (Adapter 13 to 27).

VAHTS RNA Adapters Set 3 for Illumina® (Vazyme, #N809) contains 24 adapters (Adapter 96-01 to 96-24).

VAHTS RNA Adapters Set 4 for Illumina® (Vazyme, #N810) contains 24 adapters (Adapter 96-25 to 96-48).

VAHTS RNA Adapters Set 5 for Illumina® (Vazyme, #N811) contains 24 adapters (Adapter 96-49 to 96-72).

VAHTS RNA Adapters Set 6 for Illumina® (Vazyme, #N812) contains 24 adapters (Adapter 96-73 to 96-96).

Mix thoroughly by gently pipetting up and down for 10 times.

5.2. Put the sample in a PCR instrument and run the following program for adapter ligation (Hot Lid Temperature: 105˚C):

30˚C	10 min
4˚C	Hold

Immediately proceed to the next step.

5.3. Add 5 μl of the Stop Ligation Mix to 35 μl of ligation products, mix thoroughly by gently pipetting up and down for 10 times to terminate the ligation reaction.

Note: The adapter-ligation products can be stored at 4˚C for less than 60 min.

6.Purification and Size Selection of Adapter-ligated DNA

Option A for libraries with 150-200 bp inserts (for mRNA fragmented by incubation at 94˚C for 8 min)

6A.1. Equilibrate the VAHTS DNA Clean Beads to room temperature.

6A.2. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Pipet 40 μl (1×) of beads into the above sample. Mix thoroughly by pipetting up and down for 10 times.

6A.3. Incubate at room temperature for 10 min.

6A.4. Place the sample on a magnetic stand. Wait until the soultion clarifies (about 5 min). Keep it on magnetic stand, and carefully discard the supernatant without disturbing the beads.

6A.5. Keeping the sample on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to rinse the beads. **DO NOT re-suspend the beads!** Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

6A.6. Repeat the **Step 6A.5.**

6A.7. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5-10 min.

6A.8. Take the sample out of the magnetic stand. Add 52.5 µl of Nuclease-free Water to elute DNA. Mix thoroughly by vortexing or pipetting and incubate for 2 min at room temperature. Place the tube back on the magnetic stand and wait until the soultion clarifies (about 5 min).Carefully transfer 50 µl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

6A.9. Suspend the VAHT DNA Clean Beads thoroughly by inverting or vortexing. Pipet 50 µl (1×) of the suspended beads to the product above. Mix thoroughly by pipetting up and down for 10 times.

6A.10. Incubate at room temperature for 10 min.

6A.11. Place the sample on the magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on the magnetic stand., and carefully discard the supernatant without disturbing the beads.

6A.12. Keep the sample on the magnetic stand, and add 200 µl of freshly prepared 80% ethanol to rinse the beads. **DO NOT re-suspend the beads!**Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

6A.13. Repeat the Step 6A.12.

6A.14. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5-10 min.

6A.15. Take the sample out of the magnetic stand. Add 22.5 µl of nuclease-free water to elute the DNA.Mix thoroughly by vortexing or pipetting and place for 2 min at room temperature. Place the tube on the magnetic stand and wait until the solution clarifies (about 5 min). Carefully transfer 20 µl of supernatant to a new Nuclease-free PCR tube without disturbing the beads.

Note: Immediately proceed to Step 7. Library Amplification.

Note: The dilution can be stored at -20˚C for 24 hours.

Note: DO NOT disturb the beads while drawing samples from the supernatant. Even race amount of beads will affect the quality of the final library.

Option B for libraries with > 200 bp inserts (for mRNA fragmented by incubation at 94˚C for 5 min, 85˚C for 6 min, or 85˚C for 5 min)

B-1.Purfication of ligation proucts using 1 × VAHTS DNA Clean Beads

6B.1. Equilibrate the VAHTS DNA Clean Beads to room temperature.

6B.2. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Pipet 40 µl (1×) of beads into the sample above. Mix thoroughly by pipetting up and down for 10 times.

6B.3. Incubate at room temperature for 10 min.

6B.4. Place the sample on a magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on the magnetic stand and carefully discard the supernatant without disturbing the beads.

6B.5. Keeping the sample on the magnetic stand, add 200 µl of freshly prepared 80% ethanol rinse the beads. **DO NOT** re-suspend the beads!Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

6B.6. Repeat the **Step 6B.5.**

6B.7. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5-10 min.

6B.8. Take the sample out of the magnetic stand. Add 102.5 µl of Nuclease-free Water to elute DNA. Mix thoroughly by vortexing or pipetting and incubate for 2 min at room temperature. Place the tube back on the magnetic stand. Wait until the solution clarifies (about 5 min).Carefully transfer 100 µl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

B-2.Size selection with VAHTS DNA Clean Beads

The following protocol is for a library with 350-450 bp inserts (as an example). Please refer to **Table 1** for the appropriate volume of beads for libraries with inserts of other sizes.

Table 1. Recommended conditions for bead-based size selection

Insertion Length (bp)	200 - 300	250 - 350	350 - 450	450 - 550
Library Length (bp)*	320 - 420	370 - 470	470 - 570	570 - 670
Fragmentation Condition	94℃, 5 min	85℃, 6 min	85℃, 6 min	85℃, 5 min
Volume of beads for 1st round (μl)	70 (0.7 ×)	65 (0.65 ×)	60 (0.6 ×)	55 (0.55 ×)
Volume of beads for 2nd round (μl)	10 (0.1 ×)	10 (0.1 ×)	10 (0.1 ×)	10 (0.1 ×)

*Full library length means the peak size range determined by Agilent 2100 Bioanalyzer. Library length is equal to insertion length plus adapter length(120 bp). Please refer to Step 7.4 for further information.

6B.9. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Transfer 60 μl (0.6 ×) of beads into the sample above. Mix thoroughly by pipetting up and down for 10 times.

6B.10. Incubate at room temperature for 10 min.

6B.11. Place the sample on a magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on magnetic stand and carefully transfer 155 μl of the supernatant into a new Nuclease-free PCR tube.

6B.12. Add 10 μl (0.1 ×) of VAHTS DNA Clean Beads, mix thoroughly by pipetting up and down for 10 times.

6B.13. Incubate at room temperature for 10 min.

6B.14. Place the sample on the magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on the magnetic stand and carefully discard the supernatant without disturbing the beads.

6B.15. Keeping the sample on the magnetic stand, add 200 μl of freshly prepared 80% ethanol to rinse the beads. **DO NOT re-suspend the beads!** Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

6B.16. Repeat the Step 6B.15.

6B.17. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 10 min.

6B.18. Take the sample out of the magnetic stand. Add 22.5 μl of nuclease-free water to elute DNA. Mix thoroughly by vortexing or pipetting and incubate for 2 min at room temperature. Place the tube back on the magnetic stand and wait until the solution clarifies (about 5 min). Carefully transfer 20 μl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

Note: Immediately proceed to Step 7. Library Amplification.

Note: The dilution can be storedat -20℃ for 24 hours.

Note: DO NOT disturb the beads while drawing samples from the supernatant. Even trace amount of beads will affect the quality of the final library.

7. Library Amplification

7.1. Thaw the PCR Primer Mix and Amplification Mix 1 thoroughly by inverting the tube. Prepare the reaction solution as follows:

Purified Ligation Product	20 μl	
PCR Primer Mix	5 μl	■
Amplification Mix 1	25 μl	■
Total	50 μl	

Mix thoroughly by gently pipetting up and down for 10 times.

7.2. Put the sample in a PCR instrument and run the following PCR program (Hot Lid Temperature: 105℃)

Procedure	Temperature	Time	Cycles
Pre-denaturation	98℃	30 sec	1
Denaturation	98℃	10 sec	15
Annealing	60℃	30 sec	
Extension	72℃	30 sec	
Complete Extension	72℃	5 min	1
Hold	4℃		

Note: The recommended PCR cycle number is 15, which can be adjust between 12 and 15 according to user's needs.

Note: The amplified library can be stored at 4℃ for 60 min.

7.3. Purification of the PCR product with VAHTS DNA Clean Beads.

a. Equilibrate the VAHTS DNA Clean Beads to room temperature.

b. Suspend the VAHTS DNA Clean Beads thoroughly by inverting or vortexing. Transfer 50 μl (1×) of the beads into the PCR product. Mix thoroughly by pipetting up and down for 10 times.

c. Incubate at room temperature for 10 min.

d. Place the sample on a magnetic stand. Wait until the solution clarifies (about 5 min). Keep it on the magnetic stand and carefully discard the supernatant without disturbing the beads.

e. Keeping the sample on the magnetic stand, add 200 μl of freshly prepared 80% ethanol to rinse the beads. **DO NOT re-suspend the beads!** Incubate for 30 sec at room temperature and carefully discard the supernatant without disturbing the beads.

f. Repeat the **Step e**.

g. Keep the sample on the magnetic stand, open the tube and air-dry the beads for 5-10 min.

h. Take the sample out of the magnetic stand. Add 25 μl of Nuclease-free Water to elute DNA. Mix thoroughly by vortexing or pipetting and incubate for 2 min at room temperature. Place the tube back on the magnetic stand and wait until the solution clarifies (about 5 min).Carefully transfer 22.5 μl of the supernatant to a new Nuclease-free PCR tube without disturbing the beads.

Note:The dilution can be stored at -20℃.

Note: **DO NOT** disturb the beads while drawing samples from the supernatant. Even trace amount of beads will affect the quality of the final library.

7.4. Library Quality Determination Using an Agilent Technologies 2100 Bioanalyzer.

Analyze 1 μl of purified PCR product using a Agilent DNA 1000 chip (Agilent, #5067-1504). As shownin **Fig.1**, a library with high quality should exbit a narrow peak at the expected size. A narrow peak at 128 bp suggests the contamination of adapter-dimer. To eliminate this contamination, dilute the library to 50 μl with Nuclease-free Water and repeat Step 7.3 for further purification.

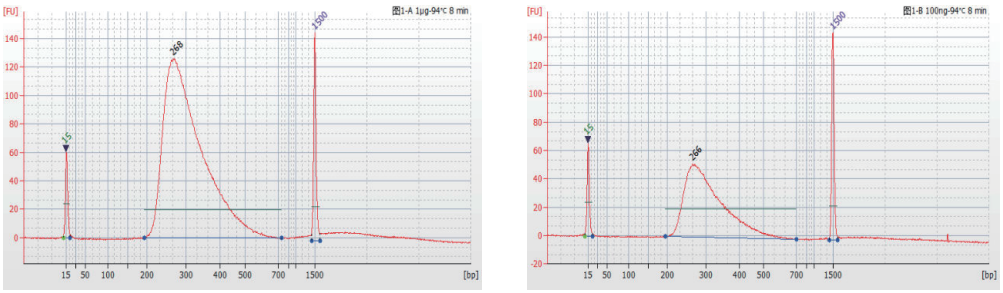


Fig1.100 ng universal human reference RNA, fragmented at 94℃ for 8 min and purified twice with VAHTS DNA Clean Beads (1×).

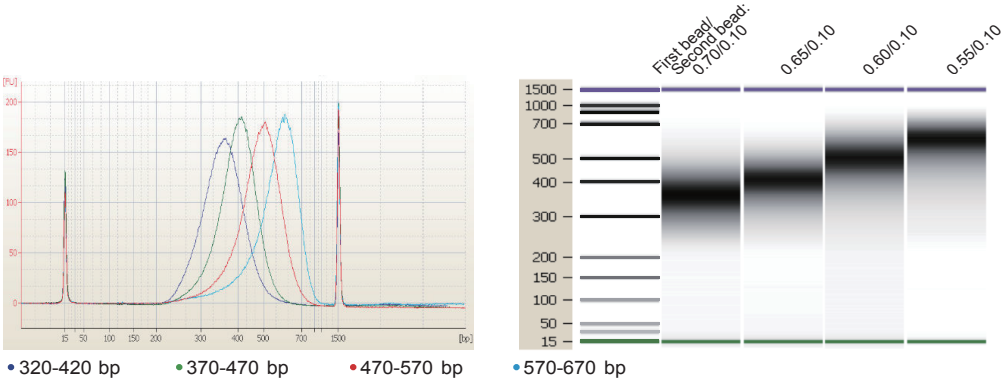


Fig1.200 ng universal human reference RNA, fragmented under 4 different conditions, and purified once with VAHTS DNA Clean Beads (1×), followed by size selection steps according to **Table 1.**

Tips

1. VAHTS DNA Clean Beads Tips

Equilibrate the beads to room temperature before use.

Mix the beads thoroughly every time before pipetting.

Thoroughly mix the beads with DNA samples.

All the DNA size selection and procedures using beads should be performed at room temperature.

Do not pipet any VAHTS DNA Clean Beads when transferring the supernatant.

Prepare fresh 80% ethanol and discard after use.

Try to remove all the 80% ethanol after washing.

Thoroughly air-dry the beads before DNA elution.

2. Avoid cross contamination between samples.

Change tips between samples.

Use filtered pipette tips.

3. Aliquot reagents after the first use to avoid repeated freeze-thaw cycles.

4. Prevent contamination of PCR products.

Isolate the experimental area and carefully clean all equipments and instruments (e.g. clean with 0.5% sodium hypochlorite or 10% bleach) to avoid possible contamination in PCR system.

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