VAHTS RNA Multiplex Oligos Set 1 for Illumina®

Catalog # N323

Version 7.1

Vazyme Biotech Co., Ltd.

Introduction

VAHTS Multiplex Oligos Set 1 for Illumina® is specially designed for RNA library preparation for Illumina® platforms. This kit contains VAHTA RNA Adapter-S for Ilumina®, 8 kinds of VAHTS i5 PCR Primers, and 12 kinds of VAHTS i7 PCR Primers. Using this kit and VAHTS Multiplex Oligos Set 2 for Illumina® (Vazyme, Cat.No.# N324) together, VAHTS mRNA-seq V3 Library Prep Kit for Illumina® (Vazyme, #NR611) provids libraries with 96 kinds of different dual-index combinations.

Contents of Kit

Appklication	Component	N323 (192 rxn)	
Universal Adapter	VAHTS RNA Adapter-S for Illumina	4 × 240 μl	
VAHTS i5 PCR Primers (RM501-RM508)	RM501	90 μl	
	RM502	90 μl	
	RM503	90 μl	
	RM504	90 μl	
	RM505	90 μl	
	RM506	90 μl	
	RM507	90 μl	
	RM508	90 μl	
VAHTS i7 PCR Primers (RM701-RM712)	RM701	60 μl	
	RM702	60 μl	
	RM703	60 μl	
	RM704	60 μl	
	RM705	60 μl	
	RM706	60 μl	
	RM707	60 μl	
	RM708	60 μl	
	RM709	60 μl	
	RM710	60 μl	
	RM711	60 μl	
	RM712	60 μl	

Storage

All the components should be stored at -20℃.

Application

Applicable for dual-indexed RNA library preparation for Illumina® platforms with VAHTS mRNA-seq V3 Library Prep Kit for Illumina® (Vazyme, Cat.No. #NR611), VAHTS Stranded mRNA-seq Library Prep Kit for Illumina® (Vazyme, Cat.No. #NR602), and VAHTS Total RNA-seq (H/M/R) Library Prep Kit for Illumina® (Vazyme, Cat.No. #NR603).

Sequence

VAHTS RNA Adapter-S for Illumina®

5′-ACACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3′

3′-CTGACCTCAAGTCTGCACACGAGAAGGCTAG-p-5′

(-s- indicates Sulfo; -p indicates Phosphorylation.)

VAHTS i5 PCR Primers

5′-AATGATACGGCGACCACCGAGATCTACAC**[i5]**ACACTCTTTCCCTACACGACGCTCTTCCGATC-s-T-3′

VAHTS i7 PCR Primers

5′-CAAGCAGAAGACGGCATACGAGAT**[i7]**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC-s-T-3′

(-s- indicates Sulfo.)

[i5] indicates i5 Index sequence (8 bp); **[i7]** indicates i7 Index sequence (8 bp).

Detailed information is as follows:

VAHTS i5 PCR Primers	Name of i5 Index	Index Sequence in Primer	Index Sequence for Sample Sheet Input/ for Sequencing	
			NovaSeq, MiSeq, HiSeq 2000/2500	MiniSeq, NextSeq, HiSeq 3000/4000
RM501	R501	TATAGCCT	TATAGCCT	AGGCTATA
RM502	R502	ATAGAGGC	ATAGAGGC	GCCTCTAT
RM503	R503	CCTATCCT	CCTATCCT	AGGATAGG
RM504	R504	GGCTCTGA	GGCTCTGA	TCAGAGCC
RM505	R505	AGGCGAAG	AGGCGAAG	CTTCGCCT
RM506	R506	TAATCTTA	TAATCTTA	TAAGATTA
RM507	R507	CAGGACGT	CAGGACGT	ACGTCCTG
RM508	R508	GTACTGAC	GTACTGAC	GTCAGTAC

VAHTS i7 PCR Primers	Name of i7 Index	Index Sequence in Primer	Index Sequence for Sample Sheet Input/ for Sequencing
RM701	R701	CGAGTAAT	ATTACTCG
RM702	R702	TCTCCGGA	TCCGGAGA
RM703	R703	AATGAGCG	CGCTCATT
RM704	R704	GGAATCTC	GAGATTCC
RM705	R705	TTCTGAAT	ATTCAGAA
RM706	R706	ACGAATTC	GAATTCGT
RM707	R707	AGCTTCAG	CTGAAGCT
RM708	R708	GCGCATTA	TAATGCGC
RM709	R709	CATAGCCG	CGGCTATG
RM710	R710	TTCGCGGA	TCCGCGAA
RM711	R711	GCGCGAGA	TCTCGCGC
RM712	R712	CTATCGCT	AGCGATAG

Protocol

Adapter Ligation

1. Bring RNA Adapter from -20℃, and mix it thoroughly after thawing.
2. Invert thawed Rapid Ligation buffer and place it on ice.
3. Combine the reaction below in a sterile PCR tube:

End Preparation Products	65 μl	
Rapid Ligation buffer	25 μl	■
Rapid DNA Ligase	5 μl	■
VAHTS RNA Adapter-S for Illumina®	2.5 μl	
Nuclease-free H_2O	2.5 μl	
Total	100 μl	

▲Firstly add VAHTS RNA Adapter-S for Illumina® to the End Preparation product, mix gently with a pipette, and then add the remaining components.

4. Mix the reaction by gently pipette for 10 times.

5. Place the reaction tube in the PCR instrument to perform ligation reaction.

Heat Lid 105°C	On
20°C	15 min
4°C	Hold

The ligation product could be store at 4°C for 1 h.

Purification and Size-selection of Ligation Product

▲ If using 1.0× VAHTS DNA Clean Beads to purify the product, the purification protocol will be the same as VAHTS mRNA-seq V3 Library Prep Kit for Illumina® (Vazyme #NR611).

Please refer to the following table if size selection is needed:

Insertion Length (bp)	200-300	250-350	350-450	450-550
Library Length (bp)	260-360	310-410	410-510	510-610
Fragmentation Condition	94°C 5 min	85°C 6 min	85°C 6 min	85°C 5 min
1st Round bead volume (μl)	80 (0.8 ×)	65 (0.65 ×)	60 (0.6 ×)	55 (0.55 ×)
2nd Round bead volume (μl)	20 (0.2 ×)	20 (0.2 ×)	10 (0.1 ×)	10 (0.1 ×)

▲ Library length here means the peak size range determined by Agilent 2100 Bioanalyzer. Library length is equal to insertion length plus adapter length (66bp).

▲ Any volume deviation of adding sample will affect the final library insert size.

▲ The volume ratio of the magnetic beads used in the sorting is relative to the initial DNA volume, for example: the initial volume of DNA is 100 μl, the volume of the first round of beads is 60 μl, which is 60% of 100 μl (0.6 ×), the volume of the second round of beads 10 μl is 10% of 100 μl (0.1 ×), instead of 10% of the 155 μl supernatant.

Library Amplification

1. Invert the thawed PCR Primer Mix and combine the reaction below in a sterile PCR tube.

Purified Ligation Product (from previous step)	20 μl	
VAHTS i5 PCR Primer RM5XX	2.5 μl	
VAHTS i7 PCR Primer RM7XX	2.5 μl	
Amplification Mix 1	25 μl	
Total	50 μl	

2. Place the reaction tube in the PCR instrument and operate under the following conditions:

Step	Temperature	Time	Cycles
Hot Lid On，105°C			
Pre-denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	
Annealing	60°C	30 sec	12-17
Extension	72°C	30 sec	
Final Extension	72°C	5 min	1
Hold	4°C		

▲ In the amount of total RNA extracted from different species and individuals, the mRNA ratio is not necessarily the same. The PCR cycles can be adjust according to the actual circumstance of species, generally for 12-17 cycles.