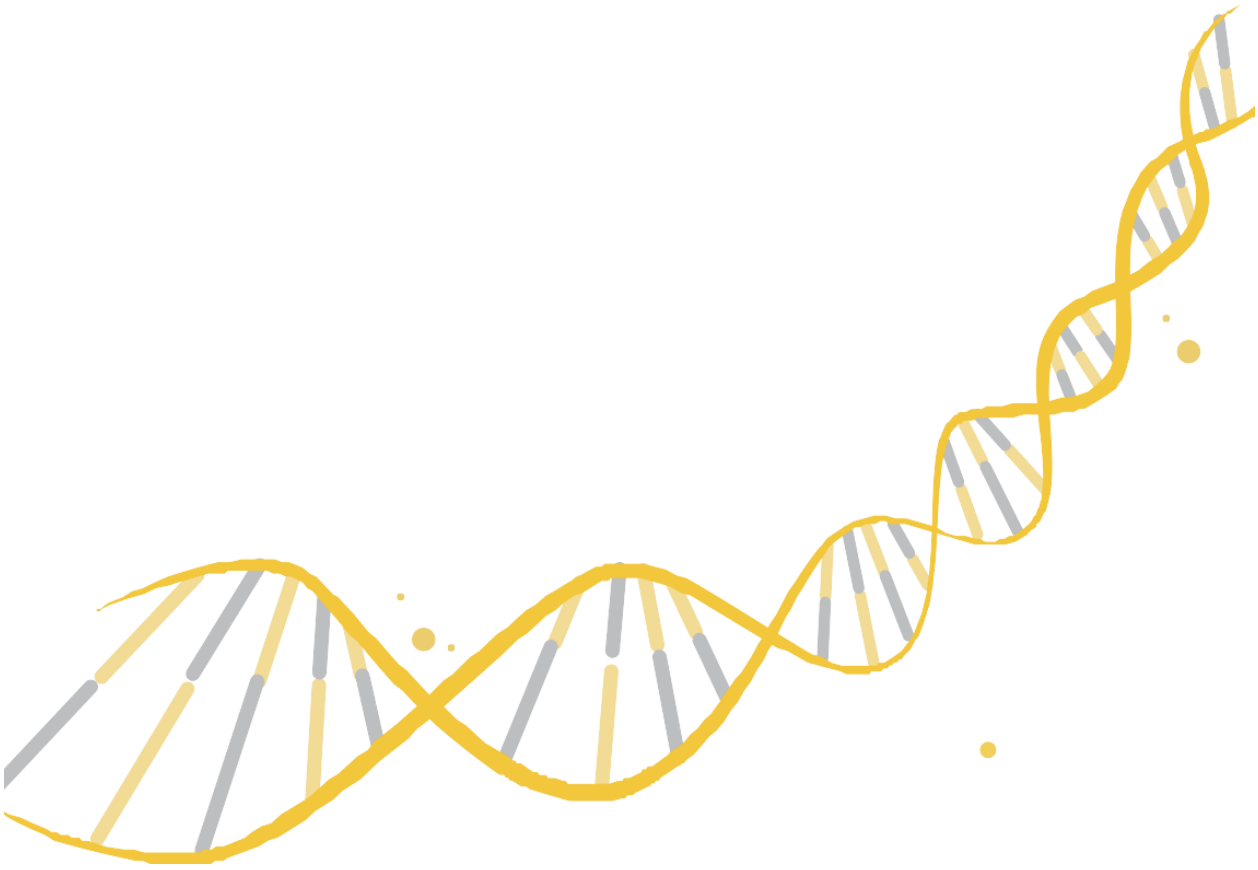
ABclonal
Whole RNA-seq Lib Prep kit for illumina RK20303



www.abclonal.com

version: NK12G02V01

Contents

1. Introduction

ABclonal Whole RNA-seq Lib Prep kit for illumina：

- Suitable for Illumina sequencing platforms;
- The rRNA depletion module (H/M/R) is mainly for human, mouse and rat total RNA, which can effectively remove cytoplasmic rRNA from total RNA (including cytoplasmic 5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA) and mitochondrial rRNA (containing 12S rRNA and 16S rRNA).
- This kit is not only suit for complete RNA samples with good integrity, but also for RNA samples that have been degraded, such as FFPE RNA. The total amount of input RNA is 10 ng to 1 ug.
- Stranded mRNA Lib Prep method is recommended as a follow strategy, Whole RNA-seq data can be used to analyze all mRNAs except ribosomes as well as some long-chain non-coding RNAs, stranded Library can quantify gene expression and RNA species more accurately, and more fully understand gene structure.
- The kit contains an RNA truncated adapter, uses RNA index primer for PCR to add a unique index to each sample. Truncated adapter has higher connection efficiency than full length adapter and can reduce the generation of Adapter dimer;
- ABclonal Whole RNA-seq Lib Prep kit for illumine contains rRNA depletion module (H/M/R), First Strand synthesis module（stranded）, Second cDNA synthesis module（stranded）, DNA Lib Prep module with UDG and ABclonal RNA index primer. Reagents and enzymes for Whole RNA-seq Library preparation are all contained.
- Each reagent in the kit has undergone rigorous quality control, and each lot of kits has been verified by Lib preparation and sequencing, ensuring stable quality performance of each lot of kits.

2. List of components

Box	Module	Name and color		24 RXN (RK20303M)	96 RXN (RK20303L)
Box-1	rRNA Depletion Module (H/M/R)（RK20348）		Probe Hybridization Buffer	48μL	192μL
			rRNA Probe Mix(H/M/R)	24μL	96μL
			RNase H	48μL	192μL
			10× RNase H Buffer	48μL	192μL
			DNase I	60μL	240μL
			10× DNase I Buffer	120μL	480μL
Box-2	First		2X Frag/Elute Buffer	264μL	1056 μL

	StrandSynthesis Module (Stranded) (RK20342)		RT Strand Specificity Reagent	192 µl	768 µl
			First Strand Synthesis Enzyme Mix	48 µl	192 µl
	Second Strand Synthesis Module (Stranded) (RK20343)		Second Strand Synthesis Reaction Buffer with dUTP	192 µl	768 µl
			Second Strand Synthesis Enzyme Mix	96 µl	384 µl
			Nuclease-free water	2 ml	8 ml
	DNA Lib Prep Module with UDG (RK20344)		End Prep Buffer	240 µl	960 µl
			End Prep Enzyme Mix	72 µl	288 µl
			Ligation Buffer	396 µl	1584 µl
			Ligase Mix	72 µl	288 µl
			2X PCR Mix	600 µl	2×1200 µl
			UDG Enzyme	12 µl	48 µl
			Low-EDTA TE	2.5 ml	10 ml
Box-3	RNA Adapter Module 24 Indices (RK20345)		RNA Truncated Adapter	60 µl	240 µl
			RNA Universal Primer	60 µl	240 µl
			RNA Index Primer	2.5 µl*	10 µl*

*RNA index primer contains 1-27, total 24 index primer. Every index primer contains 2.5 µl (24 RXN) and 10 µl (96 RXN)

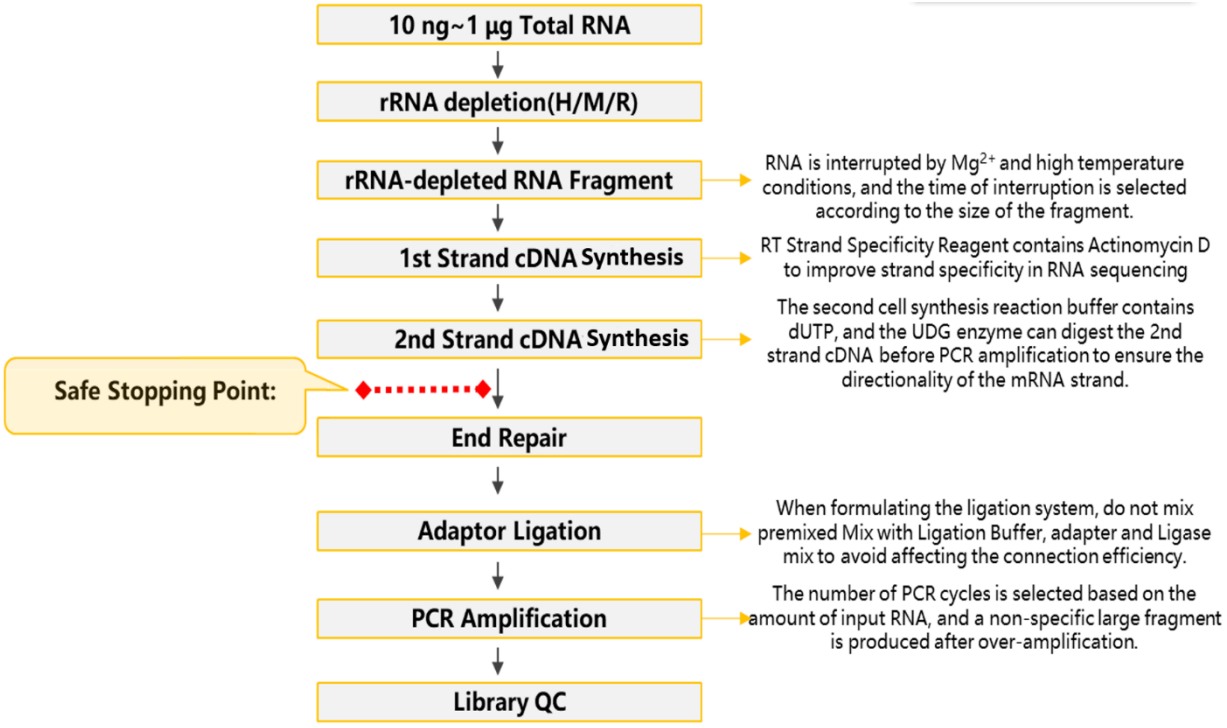
3. Storage

- Box-1: -20°C
- Box-2: -20°C
- Box-3: -20°C

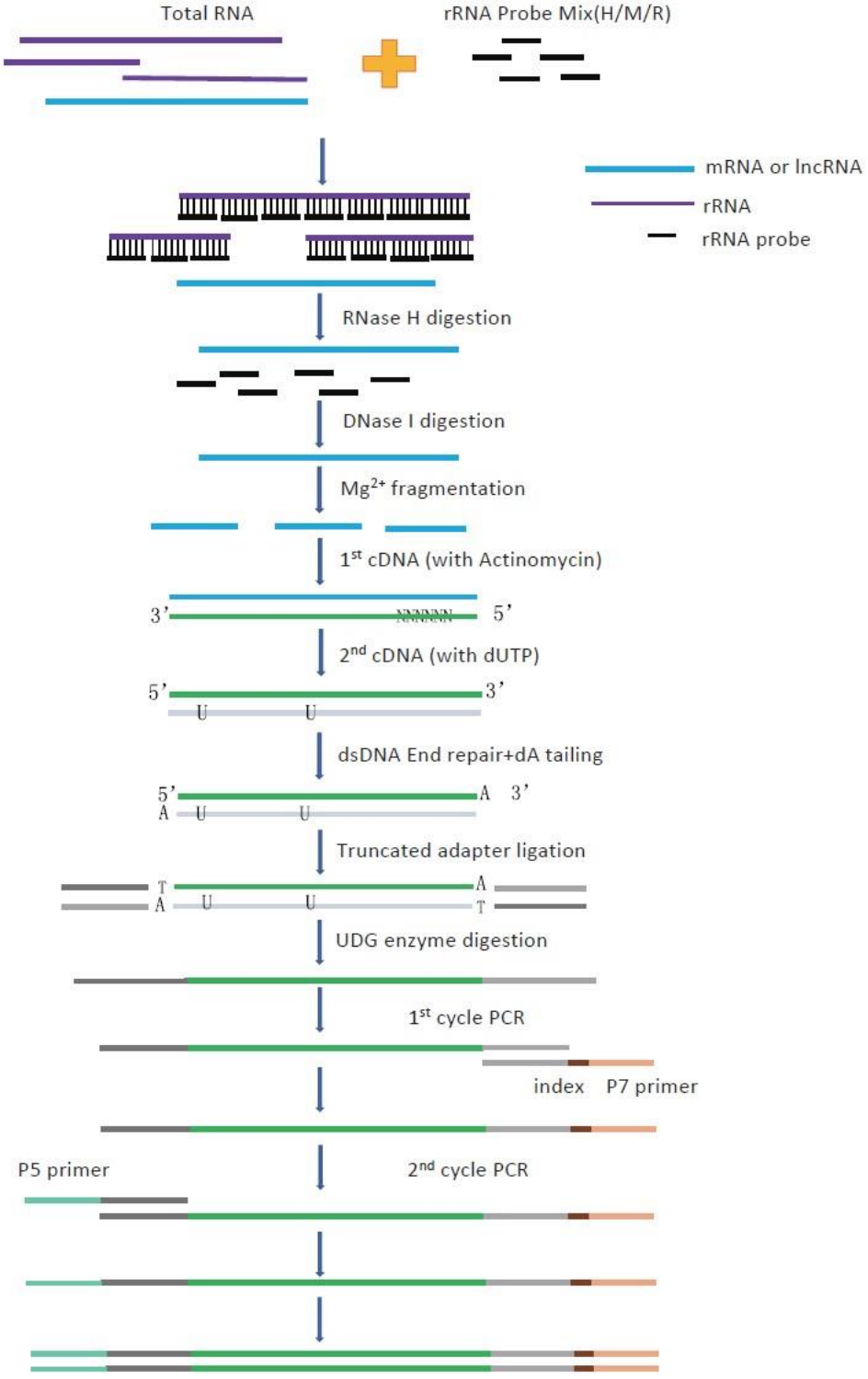
4. Additional materials Required

- Agencourt RNAClean XP beads（Beckman Coulter Inc., cat. no. A63987）
- Agencourt AMPure XP beads（Beckman Coulter Inc., cat. no. A63880）
- 100% ethanol (ACS grade)
- Magnetic stand
- Thermocycler

5. Workflow Chart



6. The Scheme of Technologies and The protocol



Precautions

- The RNA fragmentation conditions and subsequent size selection need to be selected according to the recommended range of the specification, otherwise the library size and yield will be affected.

- For FFPE RNA samples or RNA samples with severe degradation, due to poor mRNA integrity, RNA interference condition selection and fragment screening operations need to be analyzed as appropriate. Otherwise, the library yield may be affected. For details, refer to Appendix 2, FFPE. Sample or other degraded sample instructions;
- To avoid cross contamination, always carefully add the RNA index primer to the PCR reaction.

Protocol

1. rRNA depletion

1.1 Probes and rRNA hybridization

1.1.1 Dilute 10-1000 ng of total RNA with nuclease-free water to a final volume of 12 µl, put on ice.

1.1.2 Melting the Probe Hybridization Buffer on ice, and prepare the following probe hybridization pre-mix buffer:

Reagent	Volume (per sample)
Probe Hybridization Buffer	2 µl
rRNA Probe Mix(H/M/R)	1 µl
Total Volume	3 µl

1.1.3 Add 3 µl probe hybridization pre-mix buffer to the prepared 12 µl RNA and mix thoroughly by pipetting.

1.1.4 Incubate the samples in a thermocycler, carry out the hybridization. (A heated lid is set to 105°C).

Temperature	Time
95 °C	2 min
95°C-22°C	0.1 °C/sec, down to 22°C
22°C	5 min

1.1.5 After the hybridization, put the sample into ice immediately.

1.2 RNase H Digestion

1.2.1 Melting the 10× RNase H Buffer on ice, and prepare the following RNase H Digestion pre-mix buffer:

Reagent	Volume (per sample)
10× RNase H Buffer	2 µl
RNase H	2 µl
Nuclease-free H2O	1 µl
Total	5 µl

1.2.2 Add 5 µl RNase H Digestion pre-mix buffer to the prepared 1.1.5 sample to a total volume of 20 µl and mix thoroughly by pipetting.

1.2.3 Incubate the samples in a thermocycler, carry out the digestion. (A heated lid ≥45°C).

Temperature	Time
37 °C	30 min

1.2.4 After digestion,put the sample into ice immediately

1.3 DNase I digestion

1.3.1 Melting the 10×DNase I Buffer on ice, and prepare the following DNase I Digestion pre-mix buffer:

Reagent	Volume (per sample)
10×DNase I Buffer	5 µl
DNase I	2.5 µl
Nuclease-free H2O	22.5 µl
Total	30 µl

1.3.2 Add 30 µl DNase I Digestion pre-mix buffer to the prepared 1.2.4 sample to a total volume of 50 µl and mix thoroughly by pipetting.

1.3.3 Incubate the samples in a thermocycler, carry out the digestion. (A heated lid ≥45°C).

Temperature	Time
37 °C	30 min

1.3.4 After digestion, take the sample into ice immediately

1.4 rRNA-depleted RNA purification

1.4.1 Resuspend the Agencourt RNAClean XP beads by vortexing and keep on ice.

1.4.2 Add 110 µl Agencourt RNAClean XP beads(2.2X), mix thoroughly by pipetting.

1.4.3 Keep on ice for 15 min, then to a magnetic stand at RT for 15 minutes and carefully remove the supernatant.

1.4.4 Wash the beads with 200 µl of fresh 80% ethanol for 30 seconds. Pellet the beads on a magnetic stand and carefully remove the ethanol.

1.4.5 Repeat the wash step for a total of two washes.

1.4.6 Air dry the beads on a magnetic stand for 2-3 minutes. Resuspend the beads in 7 µl of Nuclease-free water and mix thoroughly by pipetting.

1.4.7 Pellet the beads and keep the tube in RT for 2 min, then on a magnetic stand for 1 min, and carefully transfer 5 µl of supernatant to a new PCR tube.

1.4.8 Adding 5µL 2×Frag/Elute buffer to the tube and mix thoroughly by pipetting. Prepare the following buffer for fragmentation

Average RNA Library Size	Fragmentation and Priming Conditions

200-300 nt	94°C 15 min, 4°C hold
300-450 nt	94°C 10 min, 4°C hold
400-700 nt	94°C 5 min, 4°C hold

Note: to FFPE samples, or degraded samples (RIN<6), could short the time, please refer to supplemental information.

1.4.9 When tubes are down to 4°C, Place the tube on ice and proceed to the first strand cDNA synthesis.

2. First Strand cDNA Synthesis

2.1 Set up the first strand cDNA synthesis reaction on ice according to the Table below.

Reagent	Volume
Fragmented and Primed mRNA	10 µl
RT Reagent	8 µl
First Strand Synthesis Enzyme Mix	2 µl
Total Volume	20 µl

2.2 Mix thoroughly by pipetting up and down several times and incubate the reaction tube in a thermocycler using the conditions listed in the Table (A heated lid is set to 105°C).

Temperature	Time
25°C	10 min
42°C	15 min
70°C	15 min
4°C	hold

3. Second Strand cDNA Synthesis

3.1 Set up the Second Strand cDNA Synthesis reaction on ice according to the Table Below

Components	Volume
First Strand cDNA Product	20 µl
Second Strand Synthesis Reaction Buffer with dUTP	8 µl
Second Strand Synthesis Enzyme Mix	4 µl
Nuclease-free Water	48 µl
Total Volume	80 µl

3.2 Incubate in a thermocycler at 16°C for 60 minutes without a heated lid.

3.3 Resuspend the AgencourtTM AMPure XP beads by vortexing and keep at RT for at least 15 minutes.

3.4 Add 144 µl (1.8X) of resuspended beads to the second strand synthesis reaction. Mix thoroughly by pipetting.

3.5 Incubate at RT for 5 minutes. Pellet the beads on a magnetic stand at RT for 5 minutes. Carefully remove and discard the supernatant.

3.6 Wash the beads with 200 µl of fresh 80% ethanol for 30 seconds. Pellet the beads on a magnetic stand and carefully remove the ethanol.

3.7 Repeat the step 3.6 for a total of two washes.

3.8 Air dry the beads on a magnetic stand for 2-3 minutes. Resuspend the beads in 39 µl of Low-EDTA TE buffer and mix thoroughly by pipetting.

3.9 Pellet the beads on a magnetic stand and carefully transfer 37 µl of supernatant to a new PCR tube.

The purified dscDNA samples can be stored at −20°C for 24 hours.

4. End Preparation of cDNA Library

4.1 Set up the end prep reaction on ice according to Table below.

Components	Volume
Second Strand Synthesis Product	37 µl
End-prep Buffer	10 µl
End-prep Enzymes Mix	3 µl
Total Volume	50 µl

4.2 Mix thoroughly by pipetting up and down several times. Incubate the samples in a thermocycler using the program listed in the Table. (A heated lid is set to 75°C).

Temperature	Time
20°C	30 min
65°C	30 min
4°C	Hold

5. Adapter Ligation

5.1 Set up the adapter ligation reaction on ice according to the Table below.

Components	Volume
End-prep DNA Product (Step 4.3)	50 µl
Ligation Buffer	16.5 µl
RNA Truncated Adapter*	2.5 µl

Ligase Mix	3 µl
Total Volume	70 µl

The truncated adapters can NOT be used for PCR-free DNA library preparation.

Note: Do NOT premix the Ligation Buffer, Ligase Mix and the RNA Truncated Adapter prior to the Adapter Ligation step.

5.2 Mix thoroughly by pipetting up and down several times. Incubate in a themocycler for 15 minutes at 22°C, without a heated lid.

6. Size selection of adapter-ligated DNA.

After adapter Ligation, there are 2 ways for purification of adapter-ligated DNA:
When input RNA < 100 ng, direct purification is recommended;
When input RNA ≥ 100 ng, follow table could provide a size selection method:

Fragmentation	**94°C 15 min**	**94°C 10 min**	**94°C 5 min**
RNA Insert Size	200-300 nt	300-450 nt	400-600 nt
Final Library Size	320-420 bp	420-570 bp	520-720 bp
1st Binding Beads ratio	0.35×	0.3×	0.25×
2nd Binding Beads ratio	0.2×	0.2×	0.15×

Direct Purification Method

6.1 Resuspend the AgencourtTM AMPure XP beads by vortexing and keep at RT for at least 15 minutes.

6.2 Add 56 µl (0.8X) of resuspended beads. Mix thoroughly by pipetting.

6.3 Incubate at RT for 5 minutes. Pellet the beads on a magnetic stand at RT for 5 minutes. Carefully remove and discard the supernatant.

6.4 Wash the beads with 200 µl of fresh 80% ethanol for 30 seconds. Pellet the beads on a magnetic stand and carefully remove the ethanol.

6.5 Repeat the wash step for a total of two washes. Resuspend the beads of 21 µl low EDTA TE. Mix thoroughly by pipetting.

6.6 Pellet the beads on a magnetic stand for 1 minute and carefully transfer 19.5 µl of supernatant to a new PCR tube.

Size Selection Method: using 94℃ 10min fragmentation as an example

6.7 Resuspend the AgencourtTM AMPure XP beads by vortexing and keep at RT for at least 15 minutes.

6.8 Adding 30 µl Nuclease-free to the Adapter Ligation Reaction system to a total volume of 100 µl.

6.9 Add 30 µl (0.3X) of resuspended beads. Mix thoroughly by pipetting.

6.10 Incubate at RT for 5 minutes. Pellet the beads on a magnetic stand at RT for 5 minutes till clarified (do not discard the supernatant).

6.11 Transfer the supernatant to a new tube, add 20 µl (0.2X) of resuspended beads. Mix thoroughly by pipetting.

6.12 Incubate at RT for 5 minutes. Pellet the beads on a magnetic stand at RT for 5 minutes. Carefully remove and discard the supernatant.

6.13 Wash the beads with 200 µl of fresh 80% ethanol for 30 seconds. Pellet the beads on a magnetic stand and carefully remove the ethanol.

6.14 Repeat the wash step for a total of two washes.

6.15 Resuspend the beads of 21 µl low EDTA TE. Mix thoroughly by pipetting.

6.16 Pellet the beads on a magnetic stand for 1 minute and carefully transfer 19.5 µl of supernatant to a new PCR tube.

7. PCR Amplification

7.1 Set up the PCR amplification reaction on ice according to the Table below.

Components	**Volume**
Purified Adapter Ligated DNA	19.5 µl
2× PCR mix	25µL
RNA Universal primer	2.5µL
RNA Index primer	2.5µL
UDG enzyme	0.5µL
Total	50µL

7.2 Mix thoroughly by pipetting up and down several times. Incubate the samples in a thermocycler using the conditions listed in the Table 11. and Table. (A heated lid is set to 105°C).

Temperature	Time	Cycles
37 ℃	10 min	1
98 ℃	1 min	1
98 ℃	10 s	8-16 *
60 ℃	15 s	
72 ℃	30 s	
72 ℃	1 min	1
4 ℃	HOLD	

*Recommended PCR cycles based on the total RNA input amount

Input Total RNA	PCR Cycles (No Size Selection)	PCR Cycles (Size Selection)
10ng	15-16	-
100ng	12-13	14-15
1µg	8-9	10-11

7.3 Resuspend the AgencourtTM AMPure XP beads by vortexing and keep at RT for at least 15 minutes.

7.4 Add 50 µl (1.0X) of resuspended beads to the PCR amplification reaction. Mix thoroughly by pipetting.

7.5 Incubate at RT for 5 minutes. Pellet the beads on a magnetic stand at RT for 5 minutes. Carefully remove and discard the supernatant.

7.6 Wash the beads with 200 µl of fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

7.7 Repeat step 7.6.

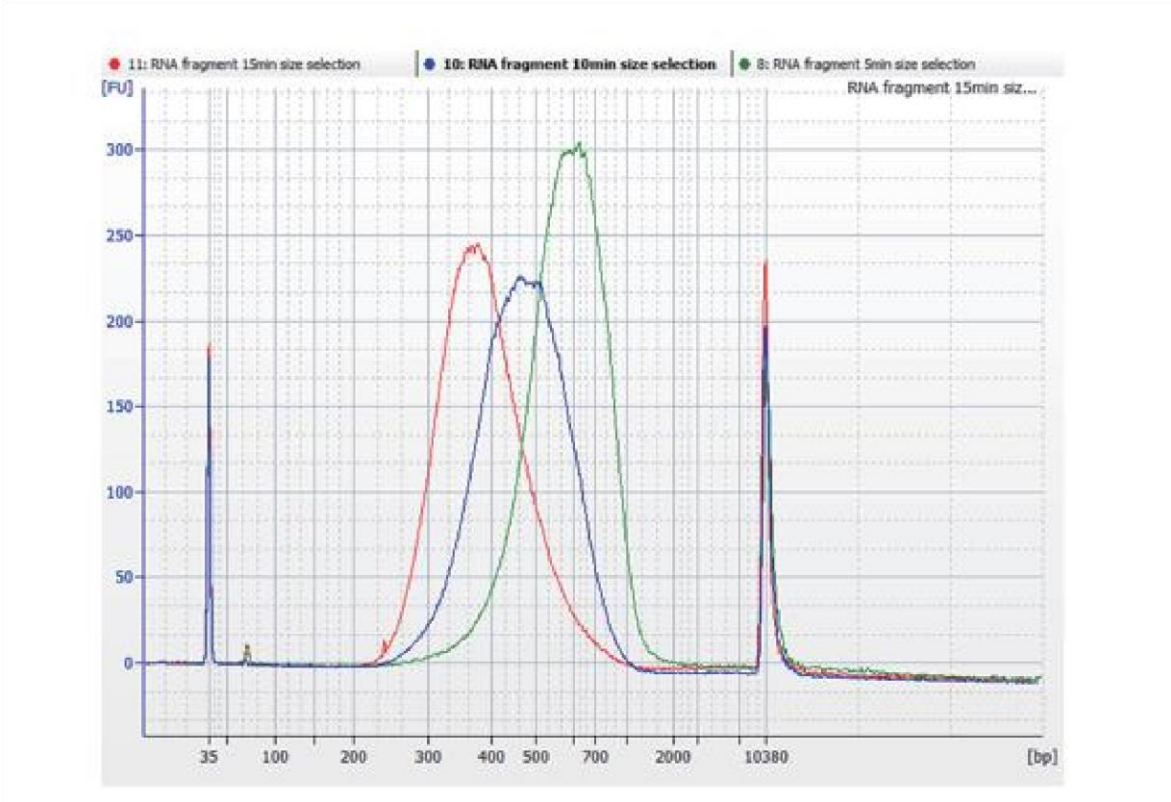
7.8 Air dry the beads on a magnetic stand for 5 minutes. Resuspend the beads in 31 µl of Low-EDTA TE buffer and mix thoroughly by pipetting.

7.9 Incubate at RT for 2 minutes. Pellet the beads on a magnetic stand and carefully transfer 30 µl of supernatant to a new PCR tube.

8 Supplemental Information

8.1 rRNA depletion library fragment distribution analysis

1ug complete (RIN>7) total RNA input, interrupted according to the recommended breaking conditions (step 1.4.8), and then selected according to the size selection procedure recommended in step 5.2. After PCR amplification the resulting library fragment size is shown in Figure 1.



The red peak: RNA fragment 15 min size selection Lib
The blue peak: RNA fragment 10 min size selection Lib
The green peak: RNA fragment 5 min size selection Lib

Figure 1: Analysis of Fragment selected library by 2100 analysis map.
1 μg of 293T cells were used to provide total RNA for input. The library was obtained by PCR 10 cycles using different fragment sorting conditions, diluted to 2 ng/μL, and analyzed by 2100 Bioanalyzer using Agilent high sensitivity DNA Chips.

8.2 FFPE or degraded RNA sample processing method

8.2.1 The mRNA integrity of the degraded samples is poor. The intact RNA breaking conditions in step 1.4.8 are not applicable to such samples. In addition, it is recommended to select the direct purification procedure after the ligation of the linker. If large-length library screening is performed, the library yield may be affected.
If you need to increase library yield, you can increase the number of PCR cycles by 2-3 cycles.

RIN value of RNA samples	Condition
>7	94℃ 15min
2-6	94℃ 7min
<2	65℃ 5min

8.2.2 FFPE sample library preparation example

When RIN=3.8

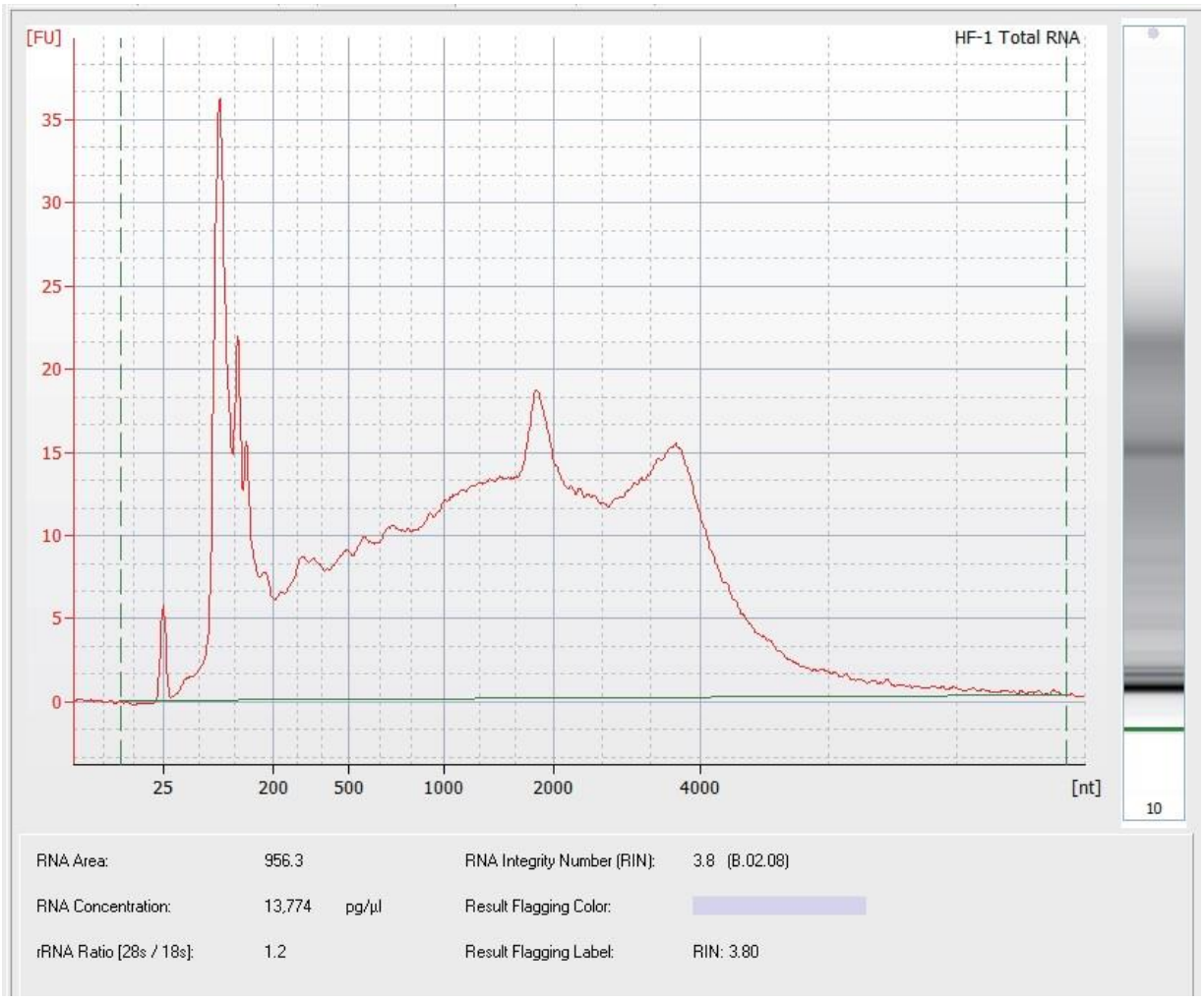


Figure 2. Analysis of FPPE RNA sample by 2100.

Human rectal cancer FFPE samples were stored at -80 °C for 1 year, and total RNA was isolated using RNeasy FFPE Kit (QIAGEN, cat. no. 73504), analyzed by Agilent Bioanalyzer RNA Pico Chips.

1. Lib Prep Without size selection

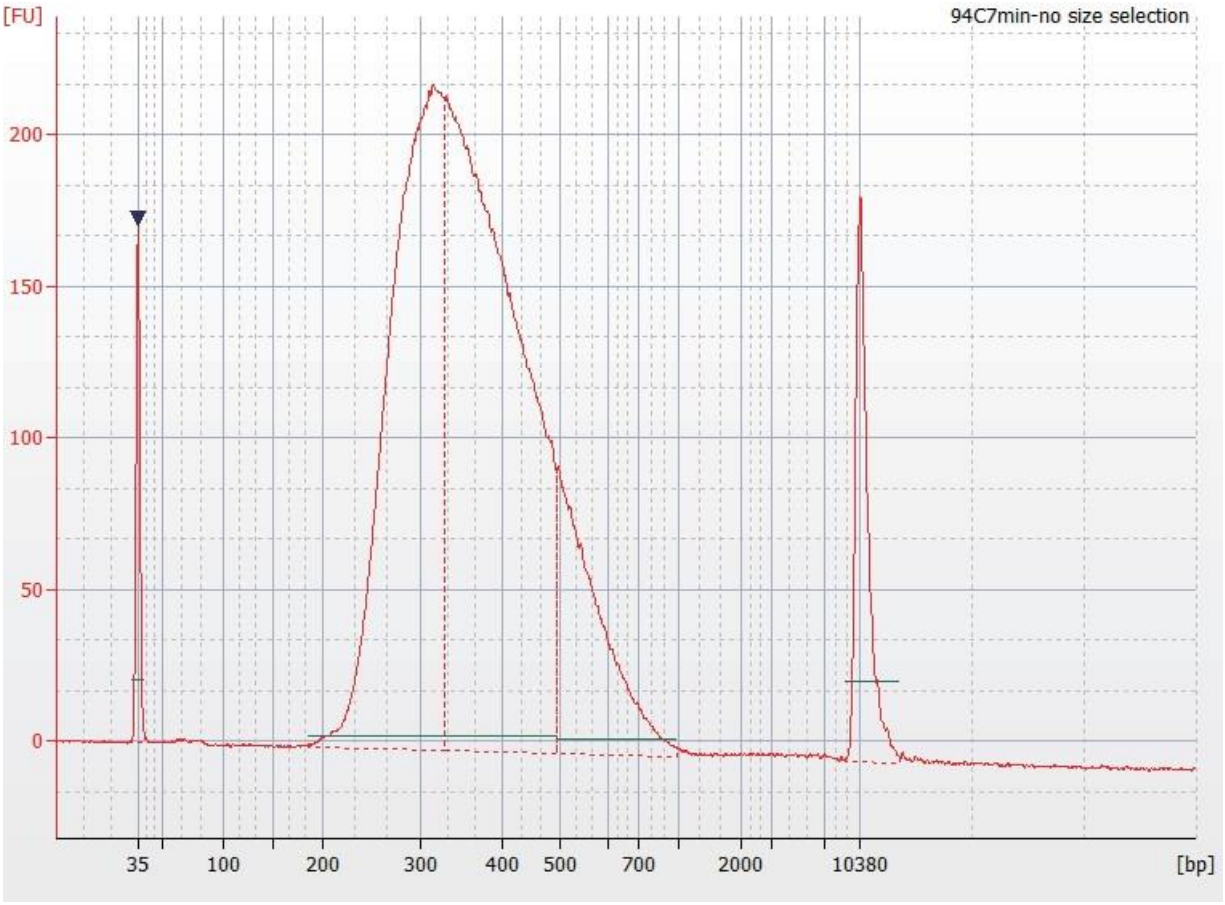


Figure 3. Analysis of FPPE RNA rRNA depletion library by 2100.
100 ng human rectal cancer FFPE RNA sample (RIN value 3.8) was injected, rRNA depletion treatment was interrupted at 94 ° C for 7 min, no fragment screening, PCR 12 cycles amplification library (18.9 ng /μL × 30 μL), the library was diluted to 2 ng / ul, Agilent Bioanalyzer High Sensitivity DNA Chip analysis

2. Lib Prep with size selection

Table 2 FFPE RNA rRNA depletion size selected library

Input RNA	Condition	Procedure for fragmentation	Peak (bp)	Cycles	Library concentration (ng/μL)	Volume (μL)
100ng	94℃ 7min	0.35×,0.2×	390bp	14	12.2	30
100ng	94℃ 5min	0.30×,0.2×	420bp	14	11.5	30
100ng	65℃ 5min	0.25×,0.15×	540bp	14	6.4	30

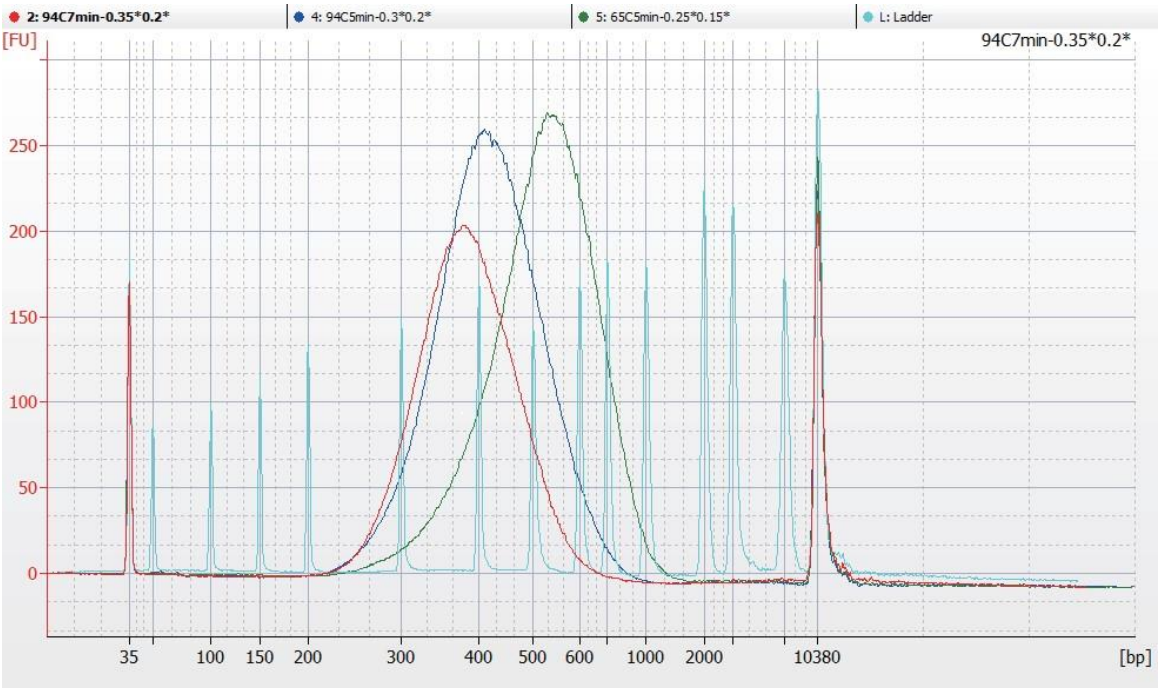


Figure 4. Analysis of FPPE RNA rRNA depletion fragment screening library by 2100. 100 ng human rectal cancer FFPE RNA sample (RIN value 3.8) input, rRNA depletion treatment, interrupted at different times, after fragment screening PCR 14 cycles amplification library, the library was diluted to 2 ng/ul and analyzed by Agilent Bioanalyzer High Sensitivity DNA Chip.

When RIN=1.9

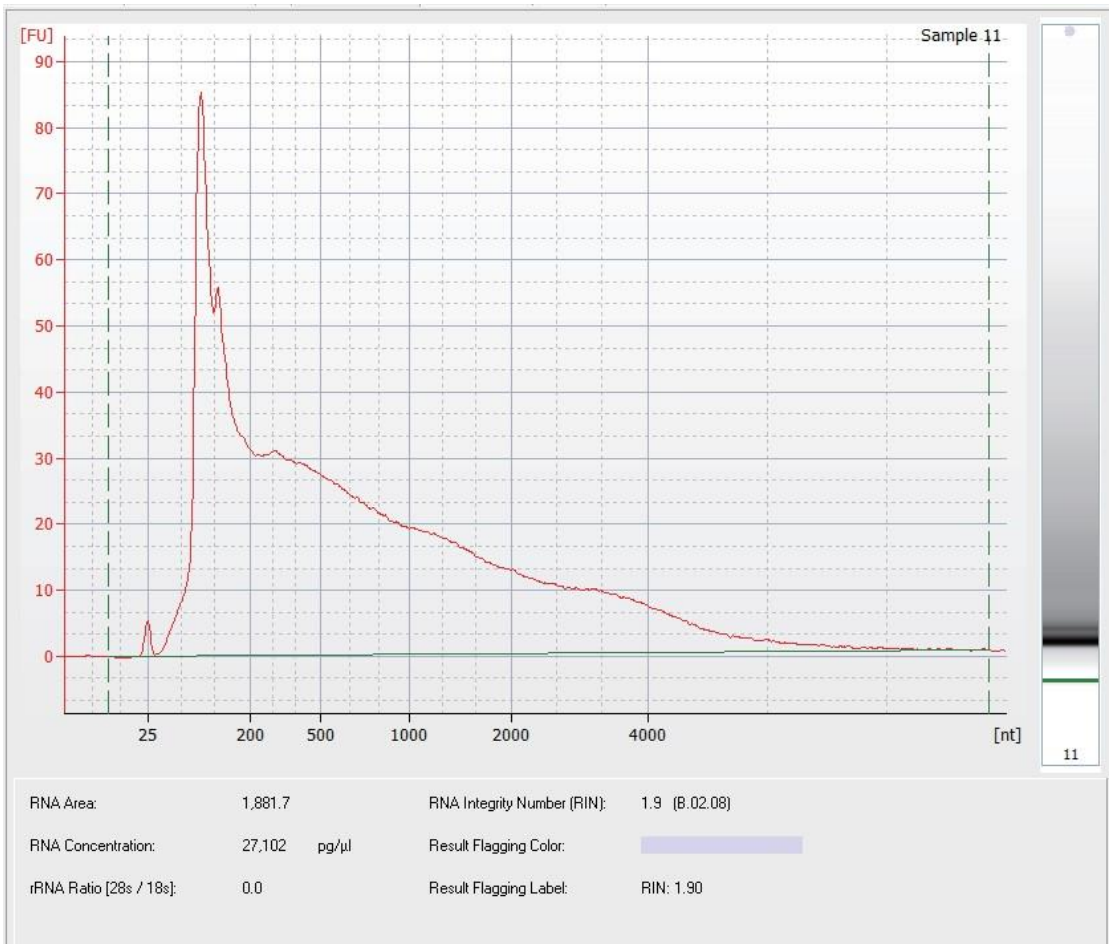


Figure 5. Analysis of FPPE RNA sample by 2100..

Mouse tissue FFPE samples were stored at room temperature for 1 year, and total RNA was isolated using RNeasy FFPE Kit (QIAGEN, cat. no. 73504), analyzed by Agilent Bioanalyzer RNA Pico Chip.

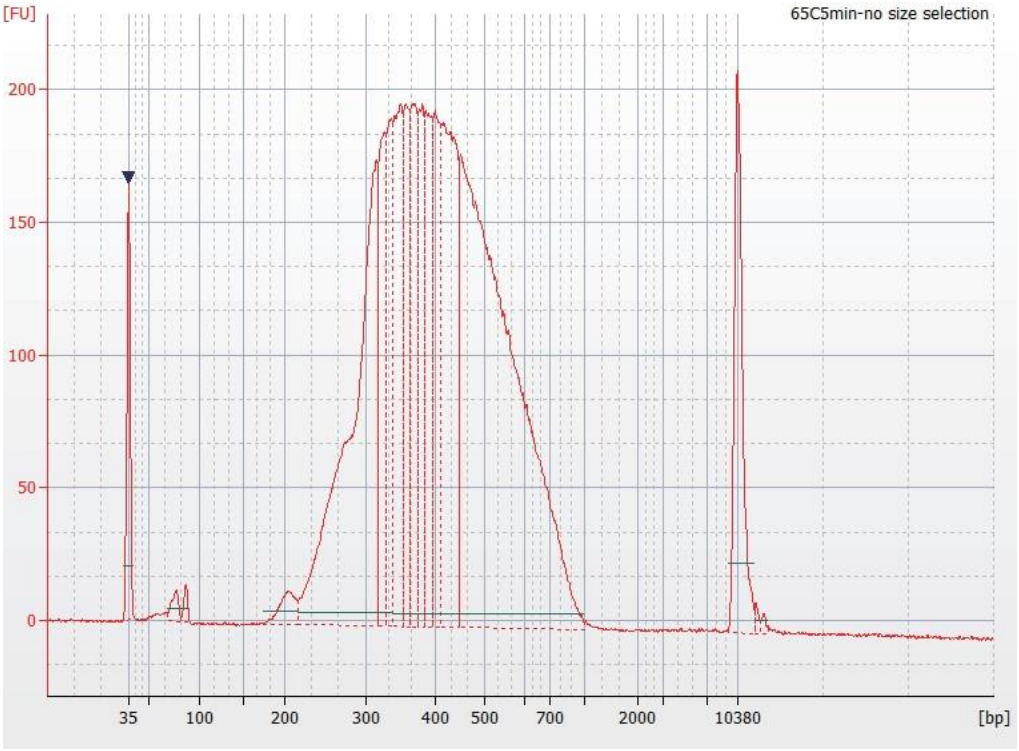


Figure 6. Analysis of FPPE RNA rRNA depletion fragment screening library by 2100.. 100ng mouse tissue FFPE RNA sample (RIN value 1.9) input, rRNA depletion treatment without 94 °C interrupt treatment, 65 ° C treatment for 5 min, no fragment screening, PCR 12 cycles expansion A library (4.34 ng/μL x 30 μL) was added and the library was diluted to 2 ng/ul, analyzed by Agilent Bioanalyzer High Sensitivity DNA Chip.

The Sequences of Adapter and Index Primers Used in the Kit

Truncated Adaptor:

5'-Spc/A*C*A*CTCTTTCCCTACACGACGCTCTTCCGA*T*C*T-3'

3'-Spc/CTGACCTCAAGTCTGCACACGAGAAGGC*T*A*G/Phos-5'

Universal PCR Primer

5'Spc/A*A*T*GATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGA*T*C*T-3'

RNA Index 1 Primer (ATCACG):

5'Spc/C*A*A*GCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGA*T*C*T-3'

Index Table: RNA Adapter module 24 index (RK20345)

RNA Index Primer name	Index	RNA Index Primer name	Index
RNA_Index_1_Primer	ATCACG	RNA_Index_13_Primer	AGTCAA
RNA_Index_2_Primer	CGATGT	RNA_Index_14_Primer	AGTTCC
RNA_Index_3_Primer	TTAGGC	RNA_Index_15_Primer	ATGTCA
RNA_Index_4_Primer	TGACCA	RNA_Index_16_Primer	CCGTCC
RNA_Index_5_Primer	ACAGTG	RNA_Index_18_Primer	GTCCGC
RNA_Index_6_Primer	GCCAAT	RNA_Index_19_Primer	GTGAAA
RNA_Index_7_Primer	CAGATC	RNA_Index_20_Primer	GTGGCC
RNA_Index_8_Primer	ACTTGA	RNA_Index_21_Primer	GTTTCG
RNA_Index_9_Primer	GATCAG	RNA_Index_22_Primer	CGTACG
RNA_Index_10_Primer	TAGCTT	RNA_Index_23_Primer	GAGTGG
RNA_Index_11_Primer	GGCTAC	RNA_Index_25_Primer	ACTGAT
RNA_Index_12_Primer	CTTGTA	RNA_Index_27_Primer	ATTCCT

Index Table: RNA Adapter module 96 index (RK20351/ RK20352)

RNA Index Primer name	Index	RNA Index Primer name	Index
RNA PCR index96 Primer 1	TTACCGAC	RNA PCR index96 Primer 25	TTCCAGGT
RNA PCR index96 Primer 2	AGTGACCT	RNA PCR index96 Primer 26	TCATCTCC
RNA PCR index96 Primer 3	TCGGATTC	RNA PCR index96 Primer 27	GAGAGTAC
RNA PCR index96 Primer 4	CAAGGTAC	RNA PCR index96 Primer 28	GTCGTTAC
RNA PCR index96 Primer 5	TCCTCATG	RNA PCR index96 Primer 29	GGAGGAAT
RNA PCR index96 Primer 6	GTCAGTCA	RNA PCR index96 Primer 30	AGGAACAC
RNA PCR index96 Primer 7	CGAATACG	RNA PCR index96 Primer 31	CAGTGCTT
RNA PCR index96 Primer 8	TCTAGGAG	RNA PCR index96 Primer 32	CTTGCTAG
RNA PCR index96 Primer 9	CGCAACTA	RNA PCR index96 Primer 33	TGGAAGCA
RNA PCR index96 Primer 10	CGTATCTC	RNA PCR index96 Primer 34	AGCTAAGC
RNA PCR index96 Primer 11	GTACACCT	RNA PCR index96 Primer 35	GAACGGTT
RNA PCR index96 Primer 12	CGGCATTA	RNA PCR index96 Primer 36	GGAATGTC
RNA PCR index96 Primer 13	TCGTCTGA	RNA PCR index96 Primer 37	TACGGTCT
RNA PCR index96 Primer 14	AGCCTATC	RNA PCR index96 Primer 38	CCAGTATC
RNA PCR index96 Primer 15	CTGTACCA	RNA PCR index96 Primer 39	TCTACGCA
RNA PCR index96 Primer 16	AGACCTTG	RNA PCR index96 Primer 40	GTAACCGA
RNA PCR index96 Primer 17	AGGATAGC	RNA PCR index96 Primer 41	GACGTCAT
RNA PCR index96 Primer 18	CCTTCCAT	RNA PCR index96 Primer 42	CTTACAGC
RNA PCR index96 Primer 19	GTCCTTGA	RNA PCR index96 Primer 43	TCCATTGC
RNA PCR index96 Primer 20	TGCGTAAC	RNA PCR index96 Primer 44	AGCGAGAT
RNA PCR index96 Primer 21	CACAGACT	RNA PCR index96 Primer 45	CAATAGCC
RNA PCR index96 Primer 22	TTACGTGC	RNA PCR index96 Primer 46	AAGACACC
RNA PCR index96 Primer 23	CCAAGGTT	RNA PCR index96 Primer 47	CCAGTTGA
RNA PCR index96 Primer 24	CACGCAAT	RNA PCR index96 Primer 48	TGGTGAAG

RNA Index Primer name	Index	RNA Index Primer name	Index
RNA PCR index96 Primer 49	AAGACCGT	RNA PCR index96 Primer 73	TAGGAGCT
RNA PCR index96 Primer 50	TTGCGAGA	RNA PCR index96 Primer 74	CGAATTGC
RNA PCR index96 Primer 51	GCAATTCC	RNA PCR index96 Primer 75	GTCCTAAG
RNA PCR index96 Primer 52	GAATCCGT	RNA PCR index96 Primer 76	CTTAGGAC
RNA PCR index96 Primer 53	CCGCTTAA	RNA PCR index96 Primer 77	TCCACGTT
RNA PCR index96 Primer 54	TACCTGCA	RNA PCR index96 Primer 78	CAACACAG
RNA PCR index96 Primer 55	GTCGATTG	RNA PCR index96 Primer 79	GCCTTAAC
RNA PCR index96 Primer 56	TATGGCAC	RNA PCR index96 Primer 80	GTAAGGTG
RNA PCR index96 Primer 57	CTCGAACA	RNA PCR index96 Primer 81	AGCTACCA
RNA PCR index96 Primer 58	CAACTCCA	RNA PCR index96 Primer 82	CTTCACTG
RNA PCR index96 Primer 59	GTCATCGT	RNA PCR index96 Primer 83	GGTTGAAC
RNA PCR index96 Primer 60	GGACATCA	RNA PCR index96 Primer 84	GATAGCCA
RNA PCR index96 Primer 61	CAGGTTCA	RNA PCR index96 Primer 85	TACTCCAG
RNA PCR index96 Primer 62	GAACGAAG	RNA PCR index96 Primer 86	GGAAGAGA
RNA PCR index96 Primer 63	CTCAGAAG	RNA PCR index96 Primer 87	GCGTTAGA
RNA PCR index96 Primer 64	CATGAGCA	RNA PCR index96 Primer 88	ATCTGACC
RNA PCR index96 Primer 65	GACGAACT	RNA PCR index96 Primer 89	AACCAGAG
RNA PCR index96 Primer 66	AGACGCTA	RNA PCR index96 Primer 90	GTACCACA
RNA PCR index96 Primer 67	ATAACGCC	RNA PCR index96 Primer 91	GGTATAGG
RNA PCR index96 Primer 68	GAATCACC	RNA PCR index96 Primer 92	CGAGAGAA
RNA PCR index96 Primer 69	GGCAAGTT	RNA PCR index96 Primer 93	CAGCATAC
RNA PCR index96 Primer 70	GATCTTGC	RNA PCR index96 Primer 94	CTCGACTT
RNA PCR index96 Primer 71	CAATGCGA	RNA PCR index96 Primer 95	CTTCGGTT
RNA PCR index96 Primer 72	GGTGTACA	RNA PCR index96 Primer 96	CCACAACA