Rapid Plus DNA Lib Prep Kit for Illumina V2

RK20255

www.abclonal.com

Version：N15F03v2.3

Contents

1. Introducton

Rapid Plus DNA Lib Prep Kit for Illumina®V2 (Cat. # RK20255) is designed to provide up to 96 indexed libraries for high multiplexing capabilities on Illumina® NGS platforms. The Kit contains all enzymes and buffers required to convert a broad range of input amounts of DNA into high quality libraries for next-generation sequencing on Illumina® NGS platforms. Inputs of 1 ng to 1000 ng double-stranded DNA (dsDNA) are required for library preparation. The entire four-step workflow takes place in a single reaction tube or well and is complete in about three hours (Figure1). No intermediate purification steps or sample transfers are necessary, thus preventing handling errors and loss of valuable samples.

Rapid Plus DNA Lib Prep Kit for Illumina® V2 includes the DNA Repair Enzymes ll components. The DNA Repair Enzymes ll components are designed to repair damaged DNA, mending the following damage types: nicks, gaps, oxidized bases, damaged/blocked 3 ’ ends, AP sites(apurin ic/apyri midinic sites), and uracil bases.

Pairing the Rapid Plus DNA Lib Prep Kit for Illumina® V2 with the Truncated DNA Adapter Kit for Illumina® Set_C/Set_D adds the capability to multiplex up to 96 NGS-ready libraries. Once purified and quantified, the resulting libraries are ready for Illumina® NGS instruments using standard Illumina® sequencing reagents and protocols. The kit provides excellent results for high-coverage deep sequencing, such as de novo sequencing, whole genome resequencing, whole exome sequencing, and other enrichment techniques.

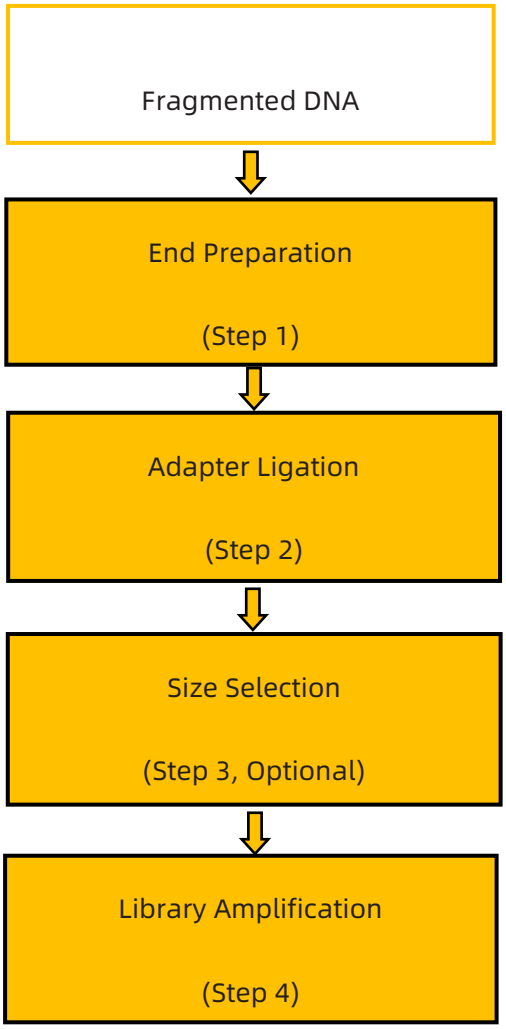

Figure 1. The workflow illustrates the procedures involved in the Rapid Plus DNA Lib Prep Kit V2. The Rapid Plus DNA Lib Prep Kit for Illumina® V2 workflow consists of three simple steps that take place in the same PCR tube or well and eliminates the need to purify and transfer sample material.

2. List of Components

All components should be stored at -20°C. The shelf lives of all reagents are one year when stored properly.

Table 1. Kit Contents

	Tube name	8 RXN	24 RXN	96 RXN
End Preparation	End Prep Buffer II	56 μL	168 μL	672 μL
	End Prep Enzymes	24 μL	72 μL	288 μL
Adapter Ligation	Damaged DNA Repair Enzymes II	24 μL	72 μL	288 μL
	Ligation Buffer	240 μL	720 μL	2880 μL
	Ligase Enzymes II	80 μL	240 μL	960 μL
Amplificaton	Gloria Nova HS 2X PCR Mix for NGS	200 μL	600 μL	2400 μL
	Illumina®PCR Primer Mix	40 μL	120 μL	480 μL

3.Additonal Materials Required

100% ethanol (80% ethanol needs to be prepared for immediate use)
Nuclease-free water
PCR strips, tubes, or plates
Magnetic stand
Pipette tip with filter element
Thermocycler
Microcentrifuge
Vortex mixer
Pipetes and multchannel pipetes
Agilent Bioanalyzer or comparable method to assess the quality of DNA library
Magnetic Purification Beads：AFTMag NGS DNA Clean Beads (ABclonal Technology Co.,Ltd., cat. no. RK20257)

Multiplex adapters (cat. # RK21622-RK21627, Unique Dual Index for Illumina; RK21706-RK21709, Unique Dual Index Adapter Plate for Illumina) compatible with Illumina® platforms

4.Precautions

Make sure to use high-quality DNA samples. Heavily nicked or damaged DNA will significantly lower library yield. We recommend Qubit® or other fluorometric methods to quantify DNA input. Residual trace RNA, nucleotides, ssDNA or other contaminants will decrease the efficiency of enzymes for optimized library preparation.

5. Protocol

Step 1. End Preparation

1.1 Prepare end-preparation reaction mix in PCR tubes on ice according to the Table 2. below.

Table 2. End-preparation Reaction Setup (per sample)

Component	Volume
Fragmented DNA	X μL
End Prep Buffer II*	7 μL
End Prep Enzymes	3 μL
Damaged DNA Repair Enzymes II**	3 μL
Nuclease-free Water	Up to 60 μL
Total volume	60 μL

* Note: End Prep Buffer II color is yellow.

** For cfDNA or high-quality DNA samples, do not add Damaged DNA Repair Enzymes II (DDREs II). For FFPE or difficult samples, DDREs II is recommended; if not being used, the cycling protocol is as follows:
20°C /30min, 65°C /30min, 4°C / Hold.

1.2 Mix thoroughly by pipetting.

1.3 Incubate reaction tubes in a thermocycler according to the program listed in Table 3. (WITH heated lid set at 75°C).

Table 3. End-preparation Reaction Program

Temperature	Time
20℃	30 min
65℃	30 min
4℃	∞

Step 2. Adapter Ligation

2.1 Prepare and dilute adapters in low-EDTA TE buffer or nuclease-free water according to the instructions in the Table 4. Below.

Table 4. Adapter Dilution

Input DNA	Adapter Dilution	Adapter Concentration
1 μg-10 ng	No dilution	15 μM
< 10 ng	2-fold	7.5 μM

2.2 Prepare the ligation reaction mix in PCR tubes on ice according to the Table 5. Below.

Table 5. Ligation Reaction Setup

Component	Vomule
End Prep Reaction Mix (Step1.3)	60 μL
Ligation Buffer	30 μL
ddH_2O	5 μL
Ligase Enzymes II	10 μL
Working Adapter（**Table 4**）*	5 μL
Total volume	110 μL

Note：The water, buffer and ligase enzymes should be premixed and then added in a single pipetting step. But the working adapter need to be added sepatately. All of the following operations are then performed on ice.

*** When performing PCR-free library preparation, use full-length adapters for the ligation step instead of truncated adapters. Each sample should be ligased with a different adapter. For example, when preparing libraries for samples 1-16, user**

needs to add adapters 1-16 accordingly. The product information, Cat No., for the full-length adapters can be found in the Appendix Table 7. List of adapters for the Illumina platforms.

2.3 Incubate at 20°C for 15 minutes in a thermocycler WITHOUT a heated lid, and then hold at 4°C. (Size Selection can be carried out in this step, and the Size Selection refers to the step 3.)

2.4 Clean up ligated DNA.

2.4.1 Add 88 μL (ratio 0.8X) of AFTMag NGS DNA Clean Beads to each samples, and mix well by pipetting.

2.4.2 Incubate the mixture at room temperature (RT) for 5 minutes.

2.4.3 Pellet the beads on a magnetic stand at RT for 2 minutes.Carefully remove and discard the supernatant (careful not to disturb pelleted beads).

2.4.4 Wash the beads with 200 μL fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

2.4.5 Repeat step 2.4.4 for a total of two washes

2.4.6 Keep the PCR tube on the magnetic, use a 10 μL pipette to remove the remaining ethanol at the bottom of the tube, open the lid of the tube and dry it until no ethanol remains.

2.4.7 Resuspend the magnetic beads in 21 μL nuclease-free water for Amplification, or resuspend the magnetic beads in 51 μL nuclease-free water for double-size selection.

2.4.8 Mix thoroughly by pipetting, and then incubate at RT for 1 minute to release the DNA from the beads.

2.4.9 Pellet the beads on a magnetic stand at RT for 2 minutes.

2.4.10 Transfer 20 μL or 50 μL of the supernatant to a new PCR tube.Store the library at -20°C until ready for library quantification.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4°C/-20°C for 1-2 weeks.

Step 3.Size Selection (optional)

3.1 Guide for size selection magnetic beads.

Table 6. Ratios of AFTMag NGS DNA Clean Beads for DNA Size Selection

Median Insert Size(bp)	150~350
Library Size (bp)	250~500
1st Binding Beads	0.7X（35 μL）
2nd Binding Beads	0.2X（10 μL）

3.2 Add 1st binding beads into 50 μL of adapter ligated DNA (from step 2.4.10), according to the volume ratio described in the Table 6., and mix thoroughly by pipetting.

3.3 Incubate at RT for 5 minutes.

3.4 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully transfer the supernatant to a new PCR tube (careful not to disturb pelleted beads).

3.5 Add 2nd binding beads to the supernatant, according to the volume ratio described in the Table 6., and mix thoroughly by pipetting.

3.6 Incubate at RT for 5 minutes

3.7 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully remove and discard the supernatant.

3.8 Wash the beads with 200 μL fresh 80% ethanol. Pellet the beads on a magnetic stand and carefully remove the ethanol.

3.9 Repeat Step 3.8 for a total of two washes.

3.10 Keep the PCR tube on the magnetic, use a 10 μL pipette to remove the remaining ethanol at the bottom of the tube, open the lid of the tube and dry it until no ethanol remains.

3.11 Air dry the beads for 2 minutes on a magnetic stand. Resuspend magnetic beads in 21 μL nuclease-free water. Mix thoroughly by pipetting, and then incubate at RT for 1 minute to release the DNA from the beads.

3.12 Pellet the beads on a magnetic stand at RT for 2 minutes. Transfer 20 μL of the supernatant to a new PCR tube for amplification.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4°C/-20°C for 1-2 weeks.

Step 4. Amplification

This kit accommodates either full-length or truncated adapters suitable for the Illumina® platform. Please select the library amplification system in Table 7 and Table 8 according to adapter type.

4.1 Prepare the PCR reaction according to the Table 7 or 8.

Table 7. PCR Amplification Reaction Setup（full-length adapter）

Adapters：Cat.NO RK21706~RK21709)

Component	Volume
Adapter-Ligated DNA	20 μL
Gloria Nova HS 2X PCR Mix for NGS	25 μL
10 PCR Primer Mix	5 μL
Total volume	50 μL

Table 8. PCR Amplification Reaction Setup（truncated adapter）

Adapters: Cat.NO RK21623~RK21627）

Component	**Volume**
Adapter-Ligated DNA	20 μL
Gloria Nova HS 2X PCR Mix for NGS	25 μL
Universal PCR Primer	2.5 μL
PCR Index	2.5 μL
Total volume	50 μL

4.2 Mix thoroughly by pi petting.

4.3 Program a thermocycler according to the Table 9 below with total amplification cycles according to Table 9.

Table 9. PCR Cycles for Library Amplification

Temperature	Time	Cycles
98℃	1min	1
98℃	10 s	2-19 PCR Cycles
60℃	30 s	
72℃	30 s	
72℃	1min	1
4℃	∞	1

*Note: 1. Add an additional 1-3 amplification cycles for FFPE samples. 2. Choose the higher number of cycles for PCR amplification when using Illumina® full-length adapters.

4.4 Add 50 μl (ratio 1.0X) of AgencourtTM AMPure XP bead to each reaction tube and mix thoroughly by pi petting.

4.5 Incubate at RT for 5 minutes.

4.6 Pellet the beads on a magnetic stand at RT for 2 minutes. Carefully remove and discard the supernatant (avoid disturbing pelleted beads).

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

4.8 Repeat Step 4.7 for a total of two washes.

4.9 Keeping the PCR tube on the magnetic stand, use a 10 μl pipette to remove remaining ethanol at the bottom of the tube. With tube lid open, allow the pellet to air dry for 2 minutes.

4.10 Resuspend the magnetic beads in 31 μl of low-EDTA TE buffer. Mix thoroughly by pi petting and then incubate at RT for 1 minute to release the DNA from the beads.

4.11 Pellet the beads on a magnetic stand at RT for 2 minutes.

4.12 Transfer 20 μl of clear supernatant to a new PCR tube.

4.13 Store the library at -20℃ until ready for library quantification or sequencing.

Safe stopping point: The purified joint ligation product can be temporarily stored at 4℃/-20℃ for 1-2 weeks.

Primer Sequences

Primer 1:

5’-AATGATACGGCGACCACCGAG

Primer 2:

5’-CAAGCAGAAGACGGCATACGAG

Appendix Table

Table 7. List of adapters for the Illumina platforms

Adapters	Index	UMI/UDI	Cat. NO	Kit
Full-length	Dual	UDI	RK21706 - RK21709	Unique Dual Index Adapter Plate for Illumina
Truncated	Dual	UDI	RK21622 - RK21627	Unique Dual Index for Illumina
	Dual	UMI/UDI	RK21701 - RK21703	Unique Dual Index (with UMI) for Illumina
Truncated	Dual	UDI	RK21687 - RK21689	Truncated DNA Adapter (UDI) Kit for MGI
Truncated	Dual	UDI	RK21721 - RK21724	Truncated DNA Adapter (UDI) Plate for MGI

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