Rapid Plus DNA Lib Prep Kit V2 (No DDREs)

RK20271

www.abclonal.com

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Contents

1. Introducton

Rapid Plus DNA Lib Prep Kit V2 (No DDREs) (Cat. NO RK20271) contains the enzymes and buffers required to convert a broad range of input amounts of DNA into high quality libraries for next-generation sequencing on the 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 tube or well, in about three hours (Figure 1). No intermediate purification steps or sample transfers are necessary, thus preventing handling errors and loss of valuable samples.

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.

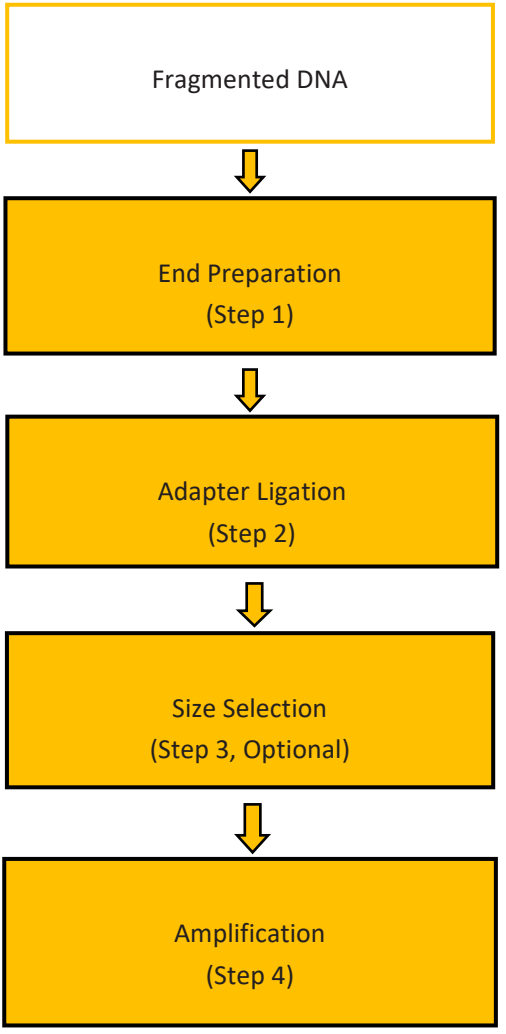

Figure 1. The workflow illustrates the processes of Rapid Plus DNA Lib Prep Kit V2 (No DDREs) .

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	End Prep Buffer II	56 μL	168 μL	672 μL
Preparation	End Prep Enzymes	24 μL	72 μL	288 μL
Adapter	Ligation Buffer	240 μL	720 μL	2880 μL
Ligation	Ligase Enzymes II	80 μL	240 μL	960 μL
	2X PCR Mix	200 μL	600 μL	2400 μL
Amplificaton	10X ILM PCR Primers	40 μL	120 μL	480 μL
	MGI 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 strip tubes or plates

Magnetc 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

Bead： AFTMag NGS DNA Clean Beads (ABclonal Technology Co.,Ltd., cat. no. RK20257)

Multplex adapters (cat. no. RK21623-RK21627) compatble with Illumina® platorms

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
Nuclease-free Water	Up to 60 μL
Total volume	60 μL

Note 1： End Prep Buffer II color is yellow.

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°C	30 min
65°C	30 min
4°C	∞

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~50 ng	No dilution	15 μM
49 ng~25 ng	2-fold	7.5 μM
24 ng~10 ng	5-fold	3 μM
9 ng~5 ng	10-fold	1.5 μM
< 5 ng	20-fold	0.75 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.

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 15. 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℃/-20℃ for 1-2 weeks.

Step 4. Amplification

This kit can use either full-length or truncated adapters compatible with the Illumina and MGI platforms. Depending on the adapter type, select the library amplification system from Table 8, Table 9, Table 10, Table 11, or Table 12. For adapter types and related product number information, refer to the attached table.

4.1 Library Amplification System

4.1.1 Illumina Platform: Prepare the following PCR reaction system:

Table 8. Illumina Library Amplification System (full-length - UDI)

Component	Volume

	Adapter-ligated DNA	20 μL
●	2X PCR Mix	25 μL
●	10X ILM PCR Primers	5 μL
	Total volume	50 μL

Table 9. Illumina Library Amplification System（trunctated - UDI）

	Component	Volume
	Adapter-ligated DNA	20 μL
●	2X PCR Mix	25 μL
○	UDI Primer	5 μL
	Total volume	50 μL

4.1.2 MG IPlatform: Prepare the following PCR reaction system

Table10. MGI Library Amplification System (full-length - Single-end index)

	Component	Volume
	Adapter-ligated DNA	20 μL
●	2X PCR Mix	25 μL
●	MGI PCR Primer Mix	5 μL
	Total volume	50 μL

Table11. Library Amplification System (truncated - Single-end Index)

	Component	Volume
	Adapter-ligated DNA	20 μL
●	2X PCR Mix	25 μL

○	MGI Index Pxxx	2.5 μL
●	MGI Universal PCR Primer	2.5 μL
	Total volume	50 μL

Table12. MGI Library Amplification System（trunctated - UDI）

	Component	Volume
	Adapter-ligated DNA	20 μL
●	2X PCR Mix	25 μL
○	MGI UDI Primers xxx	5 μL
	Total volume	50 μL

4.2 Mix thoroughly and briefly centrifuge.

4.3 Place the PCR tube in the PCR machine. The reaction program is shown in Table 13, and the recommended number of library amplification cycles is shown in Table 14. Set the PCR machine's heated lid to 105°C.

Table13. Library amplification program

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

Table14. Recommended PCR cycles

Input DNA (ng)	Yiled: 1 μg, recommended PCR cycles* (Illumina)	Yiled: 1 μg, recommended PCR cycles* (MGI)
1000	2-3	3-4
500	3-4	4-5
250	4-5	5-6
100	5-6	6-7
50	6-7	7-8
25	7-9	8-10
10	9-11	10-12
1	13-15	14-16
0.1	17-19	18-20

*Note:

1. For FFPE samples, add 1-3 additional cycles.

2. If fragment selection is performed after adapter ligation, it is recommended to add 2 more PCR cycles.

3. When using full-length adapters for the MGI platform, perform PCR amplification with the higher number of cycles.

4.4 Add 50 μL (1×) AFTMag NGS DNA Clean Beads to the amplification product, vortex to mix, and incubate at room temperature for 5 minutes.

4.5 Pellet the PCR tube on a magnetic stand for 2 minutes. When the solution is clear, carefully remove the supernatant without disturbing the beads.

4.6 Add 200 μL of 80% ethanol to wash the beads. After incubating for 30

seconds, remove the supernatant.

4.7 Repeat step 4.6.

4.8 Keep the PCR tube on the magnetic stand and use a 10 μL pipette to remove any residual ethanol from the bottom of the tube. Leave the tube open and let it air dry until no ethanol remains.

4.9 Take down the PCR tube from the magnetic stand and add 31 μL of Nuclease-Free Water to resuspend the beads. Let it stand at room temperature for 1 minute.

4.10 Pellet the PCR tube on a magnetic stand for 2 minutes, then transfer 30 μL of the supernatant to a new PCR tube.

4.11 Store the library at -20°C for library quality testing and sequencing.

Safe stopping point: Purified PCR products can be temporarily stored at 4°C for up to one week. For long-term storage, store at -20°C to avoid repeated freeze-thaw cycles.

Primer Sequences

Primer 1:

5'-AATGATACGGCGACCACCGAG

Primer 2:

5'-CAAGCAGAAGACGGCATACGAG

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