Rapid CE DNA Lib Prep Kit for Illumina® V2

RK20254

(1 ng-1 μg Input DNA)

(Illumina® Compatible)

www.abclonal.com

Version：N16A25v2.2

Contents

1. Introduction

Rapid CE DNA Lib Prep Kit for Illumina® V2 (Cat. # RK20254) is designed to provide up to 96 indexed libraries for high multiplexing capabilities on Illumina® NGS platforms. The Kit contains all the 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 tube or well and is complete in about three hours (Figure 1). No intermediate purification steps or sample transfers are necessary, thus preventing handling errors and loss of valuable samples.

Pairing the Rapid CE 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.

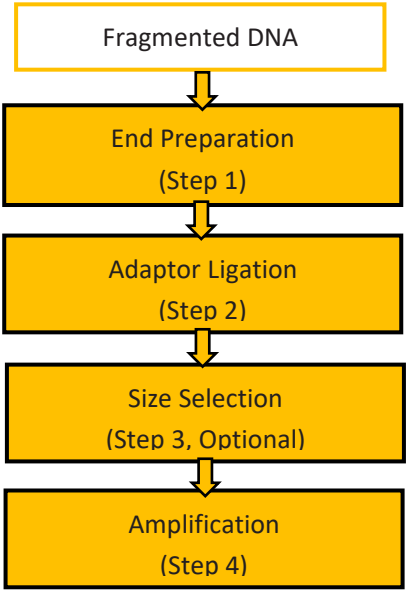

Figure 1. The following workflow illustrates the steps included in the Rapid CE DNA Lib Prep Kit V2. The Rapid CE DNA Lib Prep Kit for Illumina® V2 workflow consists of three simple steps that take place in the same PCR tube or well and eliminates the need to purify and transfer sample material.

2. List of Components

All components should be stored at -20°C. The shelf life of each reagent is one year when stored properly.

Table 1. Kit Contents

	Tube name	8 RXN	24 RXN	96 RXN
End Preparation	End Prep Buffer	80 µl	240 µl	960 µl
	End Prep Enzymes	24 µl	72 µl	288 µl
Adaptor Ligation	Ligase MM	132 µl	396 µl	1584 µl
	Ligase Mix	24 µl	72 µl	288 µl
Amplification	Gloria Nova HS 2X PCR Mix for NGS	200 µl	600 µl	2400 µl
	10X PCR Primers	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

- Pipettes and multichannel pipettes
- Agilent Bioanalyzer or comparable method to assess the quality of DNA library
- Magnetic Purification Bead：AgencourtTM AMPure XP bead (Beckman Coulter Inc., cat. no. A63880)
- Multiplex adapters (cat. # RK20292-20293, RK20294-20295) 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 the included 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 Table 2 below.

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

Component	Volume
Fragmented DNA	X µl
End Prep Buffer	10 µl
End Prep Enzymes	3 µl
Nuclease-free Water	Up to 50 µl
Total volume	50 µl

1.2 Mix thoroughly by pipetting.

1.3 Incubate reaction tubes in a thermocycler according to the program listed in Table 3. Set heated lid temperature to 75°C.

Table 3. End-preparation Reaction Program。

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

Step 2. Adaptor Ligation

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

Table 4. Adapter Dilution

Input DNA	Adaptor 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 Table 5.

Table 5. Ligation Reaction Setup

Component	Volume
End Prep Reaction Mix (Step 1.3)	50 µl
Ligation MM	16.5 µl
Ligase Mix	3 µl
Working Adaptor（**Table 4**）	2.5 µl
Total volume	72 µl

Note：The Ligation MM and Ligase Mix should be premixed and then added in a single pipetting step. Add the working adaptor separately.

2.3 Incubate at 20°C for 15 minutes in a thermocycler WITHOUT a heated lid, and then hold at 4°C.

2.4 Clean up ligated DNA. Optional Size Selection can be carried out in this procedure according to Step 3.

2.4.1 Add 57.6 µl (ratio 0.8X) of AgencourtTM AMPure XP bead to each sample 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 (avoid disturbing the 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 While keeping the sample on the magnetic stand, use a 10 µl pipette to remove remaining ethanol, then leave tube lid open to air dry the pellet for 2 minutes.

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 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 AgencourtTM AMPure XP bead 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 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 (avoid disturbing the pelleted beads).

3.5 Add 2nd Binding Beads to the supernatant according to the volume ratio described in 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 While keeping the sample on the magnetic stand, use a 10 µl pipette to remove remaining ethanol, then leave tube lid open to air dry the pellet for 2 minutes.

3.11 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. Spin down if necessary.

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 can either full-length or truncated adapters suitable for the Illumina® platform. Select the library amplification protocol according to adaptor type using Table 7, Table 8, and Table 9.

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

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

(Adapter type：Cat. # RK20292、RK20293)

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 with single index）

（Adapter type：Cat. # RK20294、RK20295）

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 pipetting.

4.3 Program a thermocycler according to Table 10 and Table 11 below and run samples through protocol.

Temperature	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

Table 10. PCR Cycles for Library Amplification

Table 11. Recommended Number of PCR Cycles

Input DNA（ng）	Number of cycles to achieve 1 ug library yield*
1000	2-3
500	3-4
250	4-5
100	5-6
50	6-7
25	7-9
10	9-11
1	13-15
0.1	17-19

*Note：1. Increase the number of amplification cycles by 1-3 with FFPE samples.

2. Choose the higher suggested cycle number when matched using full-length Illumina® adapters.

4.4 Once PCR protocol is complete, add 50 µl (ratio 1.0X) of AgencourtTM AMPure XP bead to each reaction tube and mix thoroughly by pipetting.

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 the 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 While keeping the sample on the magnetic stand, use a 10 µl pipette to remove remaining ethanol, then leave tube lid open to air dry the pellet for 2 minutes.

4.10 Resuspend the magnetic beads in 31 µl of low-EDTA TE buffer. Mix thoroughly by pipetting, 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 or until solution is entirely separated.

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

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

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

United States

www.abclonal.com

Address: 500 W. Cummings Park Dr, Woburn, MA 01801, United States

Phone: 888.754.5670, +1 857.259.4898 (Int'l)

Email: service@abclonal.com