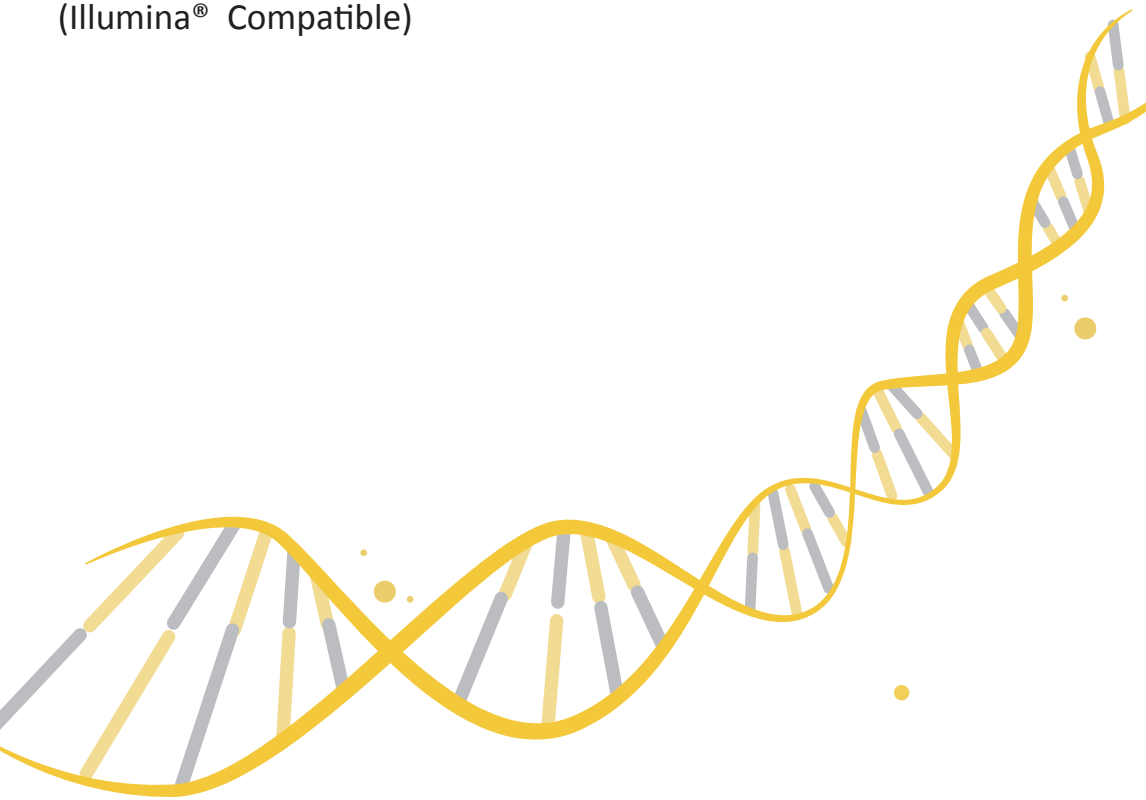
Rapid CE DNA Lib Prep kit

RK20212

(1ng-1μg Input DNA)

(Illumina® Compatible)



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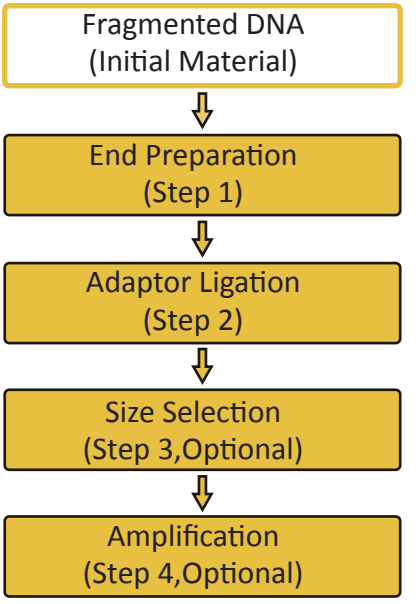
Contents

1.Introduction

The Rapid CE DNA Lib Prep kit provides a streamlined and efficient method to construct DNA library for next generation sequencing (NGS) with Illumina® platforms. The entire protocol can be completed in 2 hours. Starting from fragmented input-DNA (1 - 1000 ng), the kit contains all the enzymes and buffers for end-preparation, adapter ligation and library amplification. To simplify the workflow, the fragmented DNA is end-repaired and adenylated during the initial step. The kit can be conveniently used for construction of PCR-free libraries from 100 ng of high-quality fragmented genomic DNA, or 10 ng of circulating cell-free DNA (cfDNA). PCR primers provided in the kit are optional for users who prefer self-selected high-fidelity DNA polymerases in the library amplification.
The following workflow illustrates the processes of the Rapid DNA Lib Prep Kit.



2.List of Components

The Low-EDTA TE buffer can be stored at room temperature or 4°C. All other 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	100 µl	260 µl	1000 µl
	End Prep Enzymes	30 µl	78 µl	300 µl
Adaptor Ligation	Ligase MM	165 µl	429 µl	1650 µl
	Ligase Mix	30 µl	78 µl	300 µl
	Low-EDTA TE Buffer	1000 µl	2 mL	5 mL
Amplification	2X PCR Mix	250 µl	650 µl	2.5 mL
	10X PCR Primers	40 µl	120 µl	500 µl

3.Additional Materials Required, Not Provided

Mechanical or enzymatic methods for DNA fragmentation
Multiplex adapters (cat. no. RK20282, RK20283, RK20284, RK20285, RK20292, RK20293, RK20294, RK20295) compatible with Illumina® platforms
100% ethanol (ACS grade)
Nuclease-free water
PCR strip tubes or plates
Magnetic stand
Thermocycler
Agencourt™ AMPure XP bead (Beckman Coulter Inc., cat. no. A63880)
Pipettes and multichannel pipettes
Aerosol resistant pipette tips
Microcentrifuge
Vortex mixer
Agilent Bioanalyzer or comparable method to assess the quality of DNA library

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, ss DNA or other contaminants will decrease the efficiency of enzymes for optimized library preparation.
- Do not vortex enzymes, mix components by gently pipetting up and down several times.

5.Protocol

- Size selection step is optional for DNA libraries obtained after adapter ligation. For DNA input less than 50 ng, the size selection is not recommended.
- A PCR-based amplification step is normally required if the DNA input amount is less than 50 ng for genomic DNA and 10 ng for cfDNA.
- The full length Y-shape adapters (cat. no. RK20282, RK20283, RK20292, RK20293) suitable for Illumina® sequencing platforms are sold separately.
- If using high-fidelity DNA polymerases from other suppliers, PCR primers are included in the kit.

Step 1. End Preparation

- **Prepare fresh 80% ethanol**

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)

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 (with a heated lid at 75°C) according to the program listed in Table 3..

Table 3. End Preparation Reaction Program

20°C	30 min
65°C	30 min
4°C	∞

Step 2. Adaptor Ligation

2.1 Prepare and dilute adapters in low-EDTA TE buffer according to the instructions in the 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	1.5-fold	10 µM
24 ng-10 ng	2-fold	7.5 µM
9 ng-5 ng	5-fold	3 µM
<5 ng	10-fold	1.5 µM

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

Table 5. Ligation Reaction Setup

End Prep Reaction Mix (Step1.3)	50 µl
Ligation MM	16.5 µl
Working Adaptor	2.5 µl
Ligase Mix	3 µl
Total Volume	72 µl

2.3 Incubate the reaction at 22°C for 15 minutes in a thermocycler **WITHOUT** a heated lid, and then hold at 4°C.

2.4 Clean up ligated DNA.

2.4.1 Add 56 µl (ratio 0.8X) of Agencourt™ Ampure XP 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

2.4.4 Carefully remove and discard the supernatant.

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

2.4.6 Repeat step 2.4.5 for a total of two washes.

2.4.7 Resuspend the magnetic beads in 21 µl of Low-EDTA TE buffer.

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 of the supernatant to a new PCR tube.

2.4.11 Store the library at -20°C until ready for amplification, QC, library quantification or sequencing.

Step 3. Size Selection (optional)

Table 6. Ratios of Agencourt™ AMPure XP Beads for DNA Size Selection

Median Insert Size(bp)	250~400	300~450	400~600	500~700
Library Size (bp)	350~500	400~550	500~700	600~700
1st Binding Beads	0.35X	0.3X	0.25X	0.20X
2nd Binding Beads	0.2X	0.2X	0.15X	0.15X

3.1 Add 30 µl nuclease-free water into the tubes of ligation reaction (after step 2.3) to a total volume of 100 µl.

3.2 Add 1st binding beads to the reaction tubes, according to the volume ratio described in the Table 6., and mix thoroughly by pipetting. (e.g. For library sizes between 350 and 500 bp, the volume of binding beads should be 0.35×100 µl = 35 µl)

3.3 Incubate at RT for 5 minutes.

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

3.5 Carefully transfer the supernatant to a new PCR tube.

3.6 Add 2nd binding beads to the reaction tube, according to the volume ratio described in the Table 6., and mix thoroughly by pipetting. (e.g. For library sizes between 350 and 500 bp, the volume of binding beads here should be 0.2×100 µl = 20 µl)

3.7 Incubate at RT for 5 minutes.

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

3.9 Carefully remove and discard the supernatant.

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

3.11 Repeat Step 3.10 for a total of two washes.

3.12 Air dry the beads for 5 minutes on a magnetic stand.

3.13 Resuspend magnetic beads in 21 µl of low-EDTA TE buffer.

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

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

3.16 Transfer 20 µl of the supernatant to a new PCR tube for amplification.

Step 4. Amplification (optional)

4.1 Prepare the PCR reaction according to the Table 7. below.

Table 7. PCR Amplification Reaction Setup

Adapter-Ligated DNA	20 µl
2X PCR Mix	25 µl
10X PCR Primers	5 µl
Total Volume	50 µl

4.2 Mix thoroughly by pipetting.

4.3 Program a thermocycler according to the Table 8. below.

Table 8. PCR Cycles for Library Amplification

98°C	45 s	
98°C	10 s	
60°C	30 s	2-17 PCR cycles
72°C	30 s	
72°C	1 min	
12°C	∞	

4.4 Add 50 µl (ratio 1.0X) of Agencourt™ AMPure XP beads to each reaction tube, 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.

4.7 Carefully remove and discard the supernatant.

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

4.9 Repeat Step 4.8 for a total of two washes.

4.10 Resuspend the magnetic beads in 21 µl of low-EDTA TE buffer.

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

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

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

4.14 Store the library at -20°C until ready for QC, library quantification or sequencing.

6.Primer Sequences

Primer 1:

5’-AATGATACGGCGACCACCGAG

Primer 2:

5’-CAAGCAGAAGACGGCATACGAG

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