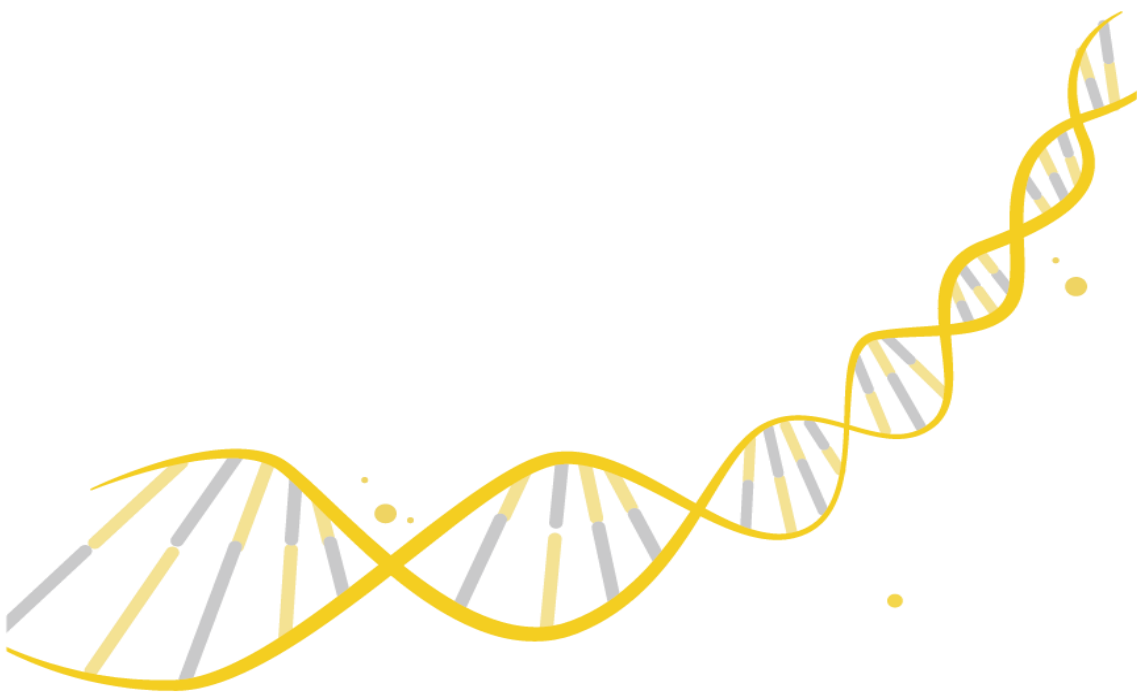


Rapid CM DNA Lib Prep Kit

RK20211



www.abclonal.com.cn

version: N14C16v1.1

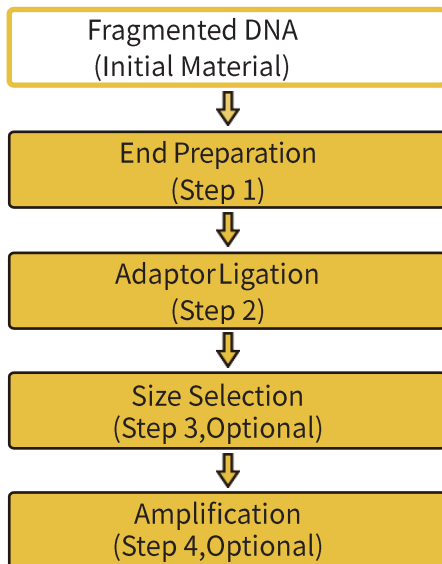
Contents

1. Introduction.....	1
2. List of Components.....	2
3. Additional Materials Required	2
4. Precautions	3
5. Protocol	3
6. Primer Sequences.....	9

1. Introduction

The Rapid CM 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, adaptor 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 CM 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 CM	30 µL	78 µL	300 µL
Adaptor Ligation	Ligase MM	165 µL	429 µL	1650 µL
	Ligase Mix CM	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

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.

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)

Components	Volume
Fragmented DNA	X μ L
End Prep Buffer	10 μ L
End Prep Enzymes CM	3 μ L
Nuclease-free Water	Up to 50 μ L
Total Volume	50 μ L

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

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

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	Adapter 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

Components	Volume
End Prep Reaction Mix (Step1.3)	50 µL
Ligation MM	16.5 µL
Working Adaptor	2.5 µL
Ligase Mix CM	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 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 μ L 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 μ L 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 μ L 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.

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
1 st Binding Beads	0.35X	0.3X	0.25X	0.20X
2 nd Binding Beads	0.2X	0.2X	0.15X	0.15X

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

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 $0.35 \times 100 \mu\text{L} = 35 \mu\text{L}$).

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 $0.2 \times 100 \mu\text{L} = 20 \mu\text{L}$).

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.

4. Amplification (optional)

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

Table 7. PCR Amplification Reaction Setup

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

4.2 Mix thoroughly by pipetting.

4.3 Program a thermocycler according to the Table 8 below.

Table 8. PCR Cycles for Library Amplification

Temperature	Time	Cycles
98°C	45 s	1
98°C	15 s	2-17 PCR cycles
60°C	30 s	
72°C	30 s	
72°C	1 min	1
12°C	∞	-

4.4 Add 50 μ L (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 μ L 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 μ L 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 μ L 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

United States

www.abclonal.com

Address: 86 Cummings Park Dr ,Woburn,MA 01801, United States

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

Email: service@abclonal.com