Rapid Plus DNA Lib Prep Kit

RK20208
RK20219 No PCR
RK20223 No DDREs

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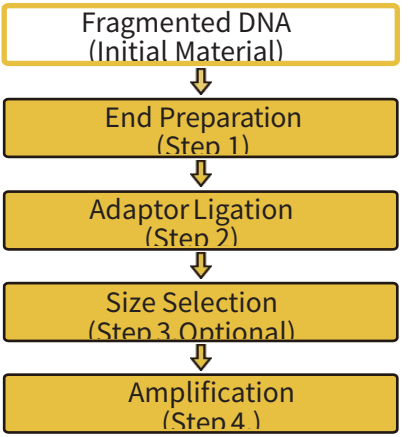
version: N16F28v2.0

Contents

1. Introduction

The Rapid Plus DNA Lib Prep kit provides a streamlined and efficient method to construction DNA library for next generation sequencing (NGS) at 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 library 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 kit also provides DNA repair enzymes, which could repair DNA damages such as nicks, gaps, oxidized bases, damaged / blocked 3'ends, AP sites (apurinic /apyrimidinic sites), and uracil bases. In addition, the DNA repair enzymes significantly increase the NGS library yields from FFPE-derived DNA. The following workflow illustrates the processes of the Rapid Plus DNA Lib Prep Kit.



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	56 µL	168 µL	672 µL
	End Prep Enzymes	24 µL	72 µL	288 µL
	Damaged DNA Repair Enzymes*	24 µL	72 µL	288 µL
Adaptor Ligation	Ligation Buffer	240 µL	720 µL	2880 µL
	Ligase Enzymes	80 µL	240 µL	960 µL
Amplification**	2X PCR Master Mix*	200 µL	600 µL	2400 µL
	10X PCR Primers*	40 µL	120 µL	480 µL

*, Note: RK20223 no contain Damaged DNA Repair Enzymes (DDREs);

**, Note: RK20219 no contain amplification reagents.

3. Additional Materials Required

Mechanical or enzymatic methods for DNA fragmentation

Multiplex adapters (cat. no. 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 and 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, ssDNA 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.

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.

For DNA input less than 10 ng, an additional cleanup step is highly recommended after ligation reaction.

The full length Y-shape adapters (cat. no. 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 µL
End Prep Buffer*	7 µL
End Prep Enzymes*	3 µL
Damaged DNA Repair Enzymes*, **	3 µL
Nuclease-free Water	Up to 60 µL
Total Volume	60 µL

*, Note: The buffer and enzyme mix should be pre-mixed and then added in a single pipetting step.

**, Note: Damaged DNA Repair Enzymes (DDREs) recommended for damaged DNA such as FFPE gDNA, mechanical fragmented DNA, optional for high quality DNA input such as cfDNA; If no DDREs addition, set up reaction program to " 20°C /30 min, 65°C /30 min, 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
30°C	30 min
65°C	30 min
4°C	∞

2. Adaptor 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

Components	Volume
End Prep Reaction Mix (Step1.3)	60 μL
H_2O*	5 μL
Ligation Buffer*	30 μL
Working Adaptor	5 μL
Ligase Enzymes*	10 μL

Total Volume	110 µL

*, Note: The water, buffer and ligase Enzymes should be premixed and then added in a single pipetting step.

2.3 Incubate the reaction 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.

2.4.1 Add 88 µL (ratio 0.8X) of AgencourtTM 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 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.

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

3. Size Selection (optional)

Table 6. Ratios of AgencourtTM AMPure XP 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)

3.1 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.

3.2 Incubate at RT for 5 minutes.

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

3.4 Carefully transfer the supernatant to a new PCR tube.

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.

3.8 Carefully remove and discard the supernatant.

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

3.10 Repeat Step 3.9 for a total of two washes.

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

3.12 Resuspend magnetic beads in 21 μL nuclease-free water.

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

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

3.15 Transfer 20 μL of the supernatant to a new PCR tube for

amplification.

4. Amplification

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

Table 7. PCR Amplification Reaction Setup

Components	Volume
Adapter-Ligated DNA	20 µL
2X PCR Master 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

Temperature	Time	Cycles
98℃	1 min	1
98℃	10 s	2-17 PCR cycles
60℃	30 s	
72℃	30 s	
72℃	1 min	1
12℃	∞	-

4.4 Add 50 µL (ratio 1.0X) of AgencourtTM 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-3’

Primer 2: 5′ -CAAGCAGAAGACGGCATACGAG-3’

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