

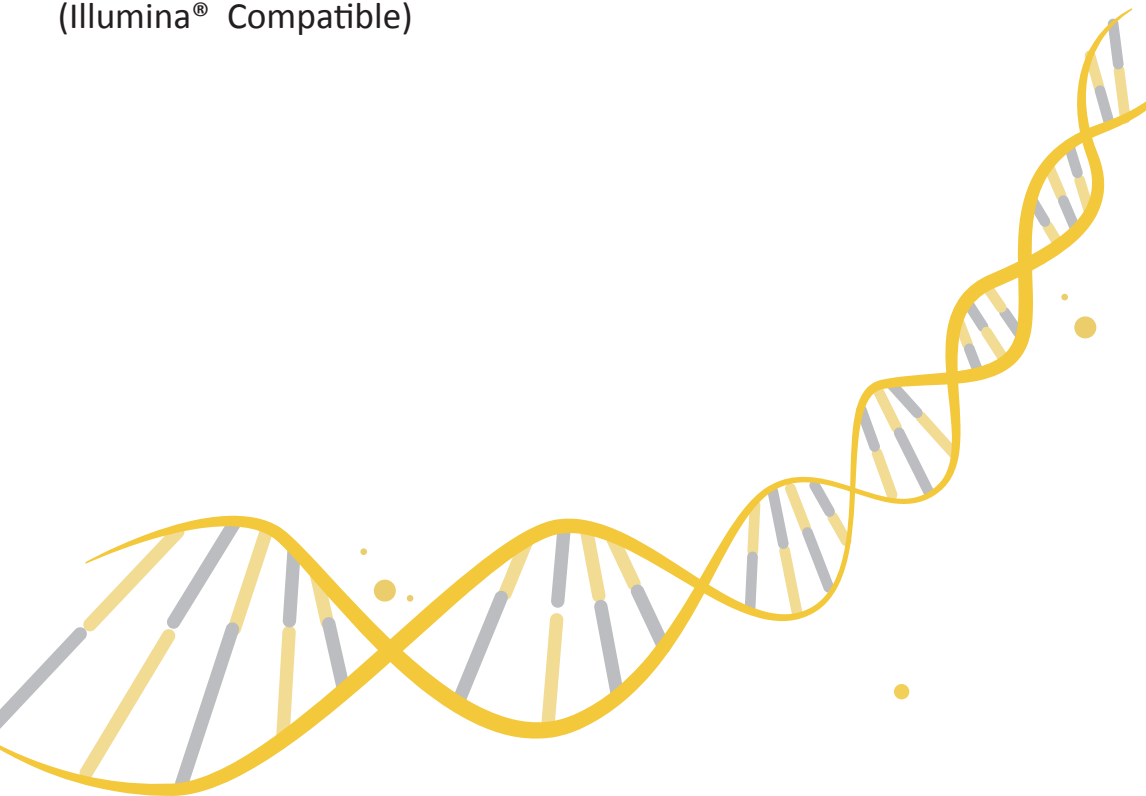


Rapid DNA Lib Prep kit

RK20200

(1ng-1 μ g Input DNA)

(Illumina[®] Compatible)



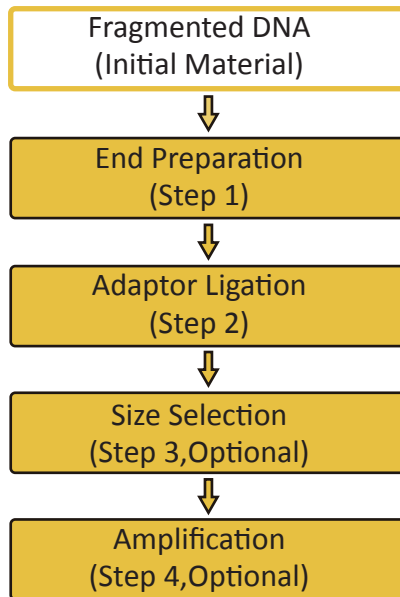
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1.Introduction

The Rapid 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 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 Master 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 μ l |
| End Prep Buffer | 10 μ l |
| End Prep Enzymes | 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

| | |
|------|--------|
| 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 |
| 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 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 $0.35 \times 100 \mu\text{l} = 35 \mu\text{l}$)

- 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.

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 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

| | | |
|------|-------|-----------------|
| 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'-CAAGCAGAAGACGGCATAACGAG

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