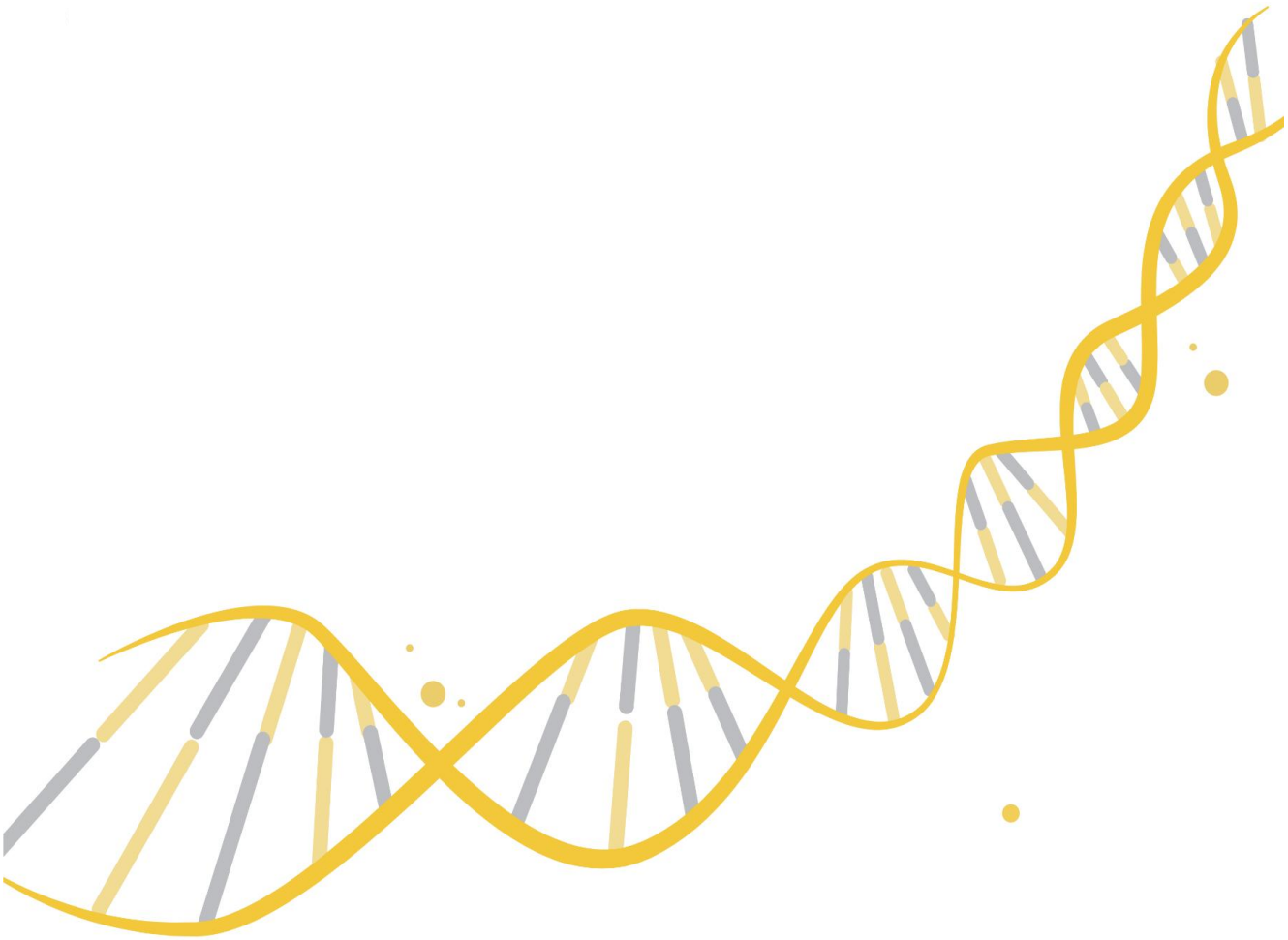
Fast WGS Reagent

RK29943



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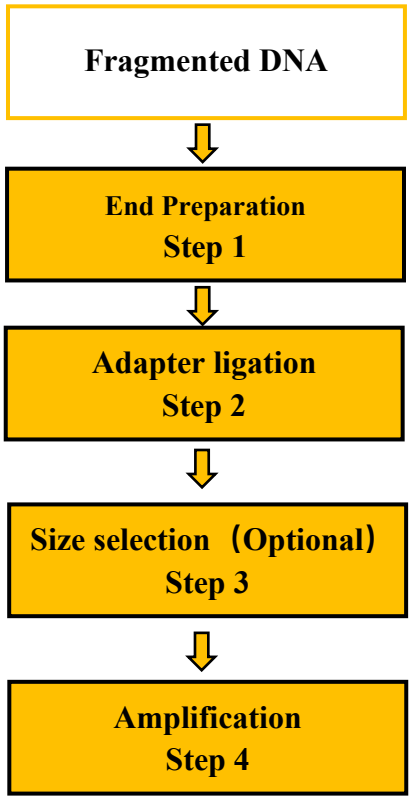
1. Product Overview

Fast WGS Reagent (Cat.#RK29943) is designed to provide up to 96 indexed libraries for high multiplexing capabilities on Illumina®NGS platforms.The Kit contains all enzymes and buffers required to convert abroad 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-strande dDNA(dsDNA) are required for library preparation.

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.

Fast WGS Reagent Work Flow



2. Components and Storage

Store all components at -20℃.

Table 1. Components

	Components	96 RXN
A1-H1	End Prep Buffer	84 μL × 8
A3-H3	End Prep Enzymes	36 μL × 8
A5-H5 A6-H6	Ligation Buffer	180 μL × 16
A8-H8	Ligase Enzymes	120 μL × 8
A10-H10	2X PCR Master Mix	300 μL × 8
A11-H11	Nuclease-free Water	12 μL × 8
A12-H12	Nuclease-free Water	60 μL × 8

3. Additional Materials Required but not Supplied

100% ethanol (80% ethanol needs to be prepared in fresh)

PCR tubes or plate; Magnetic stand

Aerosol resistant pipette tips

Thermocycler

Micro-centrifuge

Vortex mixer

Pipettes and multichannel pipettes

Agilent Bioanalyzer or comparable method to assess the quality of DNA library

AFTMag NGS DNA Clean Beads (ABclonal, Cat. RK20257) or AgencourtTM AMPure XP beads (Beckman Coulter Inc., cat. no. A63880)

Multiplex adapters compatible with Illumina® platforms（cat. no. RK20292, RK20293, RK20294）

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 Fluor metric 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.
- For genomic DNA with input less than 50 ng, PCR amplification is recommended.
- The adapters (cat. no. RK20292, RK20293, RK20294, RK20295) suitable for Illumina® sequencing platforms are sold separately.

Step 1 End Preparation

1.1 Prepare end-preparation reaction mix in sterile PCR tubes on ice according to the Table 2. below.

Table 2. End Preparation Reaction Mix (per sample)

Component	Volume
Fragmented DNA	X μL
End Prep Buffer	7 μL
End Prep Enzymes	3 μL
Nuclease-free Water	Up to 60 μL
Total Volume	60 μL

Note:

The components in the End Prep Buffer will undergo oxidation reactions when exposed to air, resulting in yellowing. During the shelf life, minor color changes caused by oxidation do not affect the performance of the reagent.

1.2 Mix thoroughly by pipetting.

1.3 Incubate reactions in a thermocycler according to the program listed in Table3. Set heated lid set temperature to 75°C.

Table 3. End Preparation Reaction Program

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

Step 2 Adapter Ligation

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

Table 4. Adapter Dilution

Input DNA	Dilution ratio	Adapter Concentration
1 μg~10 ng	No dilution	15 μM
< 10 ng	2-fold	7.5 μM

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

Table 5. Ligation Reaction Setup

Component	Volume
End Prep Reaction Mix (Step 1.3)	60 μL
Ligation Buffer	30 μL
Nuclease-free Water	5 μL
Ligase Enzymes	10 μL
Working Adapter（Table 4）	5 μL
Total Volume	110 μL

Note:

Nuclease-free Water, Ligation Buffer, Ligase Enzymes can be pre-mixed. The Working Adaptor must be added separately. Whole process need to be operated on ice.

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. Optional Size Selection can be performed during this procedure according to the protocol in Step3.

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. Carefully remove and discard the supernatant.

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 With the PCR tubes on the magnetic stand, use a 10 μL pipette to remove remaining ethanol, then leave tube lid open to pellet for 2 minutes.

2.4.7 If proceeding to **amplification**, resuspend the magnetic beads in 21 μL nuclease-free water. For **size selection**, resuspend the magnetic beads in 51 μL nuclease-free water.

2.4.8 Mix thoroughly by pipetting, then incubate at RT for 1 minute to release the DNA from the beads. Spin down if necessary.

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℃ until ready for library quantification.

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

Step 3 Size Selection (Optional)

Table 6. Ratios of Agencourt™ AMPure XP Beads

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 Guide for size selection using magnetic beads

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 the 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 disturbin gpelleted beads).

3.5 Add 2nd Binding Beads to the supernatant, according to the ratio 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 3.8 for a total of two washes.

3.10 Keeping the PCR tube on the magnetic stand, use a 10 μL pipette to remove remaining ethanol at the bottom of the tube. With tube lid open, air dry the beads for 5 minutes on a magnetic stand.

3.11 Resuspendmagneticbeadsin21μlnuclease-freewater.

3.12 Mix thoroughly by pipetting, then incubate at RT for1minute to release the DNA from the beads. Spin down if necessary.

3.13 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 accommodates either full-length or truncated adapters suitable for the Illumina ®platform. Please select the library amplification system in Table 7, Table 8, and Table 9 according to adapter type.

4.1 Prepare the PCR reaction according to the Table 7 & Table 8 below.

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

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

Table 8. PCR Amplification Reaction Setup（Truncated adapter with single index）

Component	Volume
Adapter-Ligated DNA	20 μL
2X PCR Master Mix	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 the Table 9 below with total amplification cycles according to Table 10:

Table 9. PCR programs for Library Amplification

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. Number of PCR Cycles

Input DNA (ng)	Number of PCR Cycles for 1 μg library yield*
1000	2-4
500	4-5
250	5-6
100	6-7
50	7-8
25	8-10
10	10-12
1	14-16
0.1	18-20

*Note:

1. Add an additional 1-3 amplification cycles for FFPE samples.
2. Choose the highe rnumber of cycles for PCR amplification when using Illumina®full-length adapters.

4.4 Add 50 μL (ratio 1.0X) of AgencourtTM AMPure XP beads to each reaction tubes, 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 disturbin gpelleted beads).

4.7 Wash the beads with 200 μL fresh 80% ethanol. Pellet the beads on a magnetic stand for 30s and carefully remove the ethanol.

4.8 Repeat 4.7 for a total of two washes.

4.9 Keeping the PCR tube on the magnetic stand, use a 10 μL pipette to remove remaining ethanol at the bottom of the tube. With tube lid open, allow the pellet beads to air dry for 2 minutes.

4.10 Resuspend the magnetic beads in 31 μL 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.

4.12 Transfer 30 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.

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