艾科瑞生物
Accurate Biology

Instruction for Use

AccuNext Fast Tagment DNA Library Prep Kit for Illumina (50 ng Input DNA)

AG12521
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a tagmentation-based library preparation kit specifically designed for the Illumina high-throughput sequencing platform. It enables the preparation of sequencing libraries from 50 ng of purified DNA. Using a novel transposase and an optimized buffer system, this kit allows simultaneous DNA fragmentation and adapter ligation within 10 minutes. Compared with conventional library preparation workflows, it offers a simpler and faster process by eliminating the need for time-consuming steps such as DNA fragmentation, end repair, and adapter ligation-significantly reducing library construction time.

Since the Transposome Mix V50 is highly sensitive to the input amount of DNA, accurate DNA quantification is critical for successful results. It is strongly recommended to use exactly 50 ng of DNA as input without adjustment. For other input amounts, additional kit variants are available:

100 ng (Code No. AG12518 / AG12519)
5 ng (Code No. AG12522 / AG12523)
1 ng (Code No. AG12524 / AG12525)

The reaction system of this product has been carefully optimized. All reagents used in the experiment should be those provided within the kit. It is not recommended to modify reagent volumes or concentrations, or to replace any components with equivalent products from other sources, as this may lead to suboptimal results. If replacement is necessary, validation experiments should be performed beforehand.

2. Kit Information

Kit Name	Cat. No	Specification
AccuNext Fast Tagment DNA Library Prep Kit for Illumina (50 ng Input DNA)	AG12521	48 Rxns

3. Transportation and Storage

Storage	Store at −80°C
Transportation	Transport at −20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
AccuNext Transposome Mix V50*a	48µl
5X Tn5 Reaction Buffer*b	192µl
5X Stop Buffer*b	240µl
AccuNext PCR Mix*b	1.3 ml

*a: AccuNext Transposome Mix V50 should be stored at −80°C. It is optimized for use with 50 ng of DNA template, and the input amount should not be adjusted.

*b: 5X Tn5 Reaction Buffer, 5X Stop Buffer, and AccuNext PCR Mix can be stored either at −80°C or −20°C.

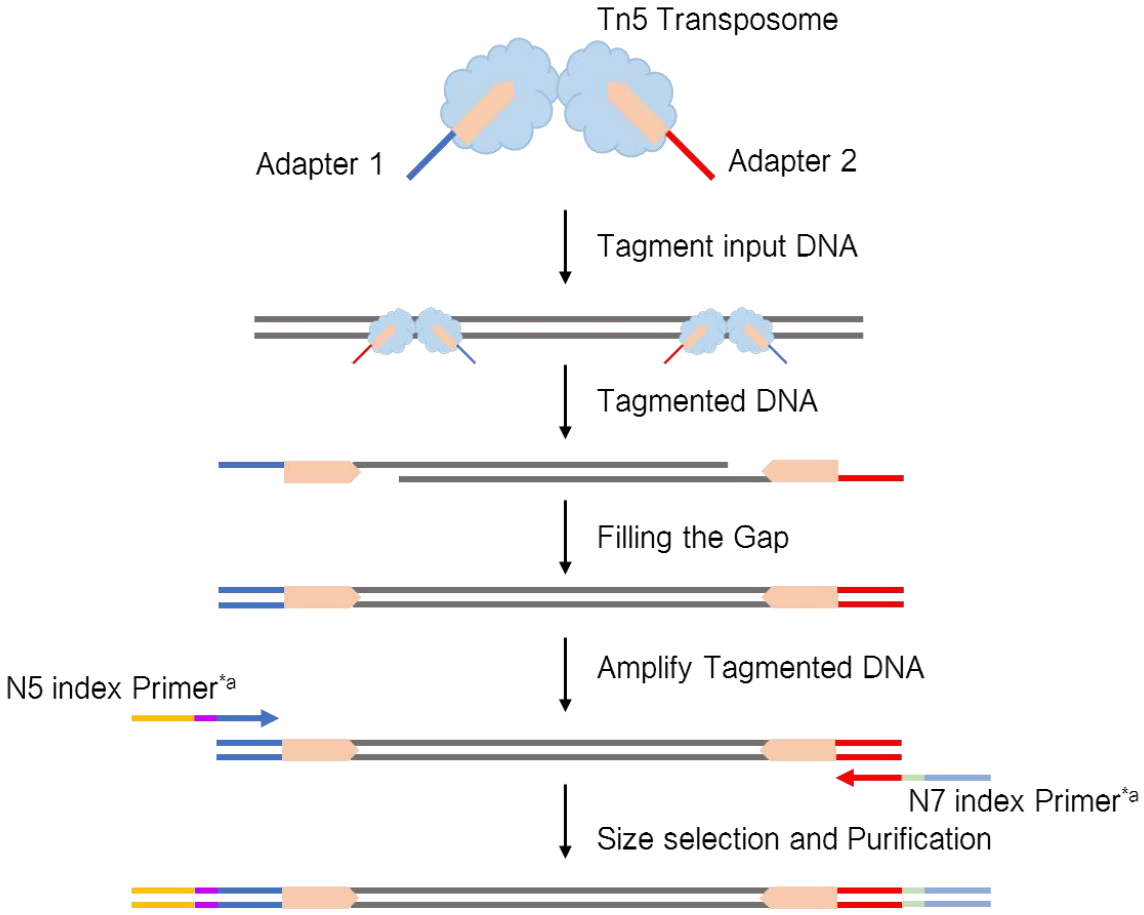
*c: The Illumina sequencing adapter primers required for PCR amplification are not included in this product and must be purchased separately. For example, AccuNext Tagmentation Library Adapter Primers (Illumina) (Code No. AG12532, AG12533, AG12534) can be used.

5. General Guideline

5.1 Experimental Principle and Workflow

AccuNext Transposome Mix consists of a transposase complexed with two adapters, Adapter 1 and Adapter 2, forming a complete transposome.During the transposition process, the transposome inserts the Adapter 1 and Adapter 2 sequences into the target DNA, generating fragments with Adapter 1 at one end and Adapter 2 at the other. Subsequently, DNA polymerase fills in the gaps created during transposition. The adapter-ligated DNA fragments are then amplified using Illumina sequencing primers N5 Index Primer and N7 Index Primer. After purification and size selection, the sequencing-ready library is obtained.

Figure 1 Experimental Principle

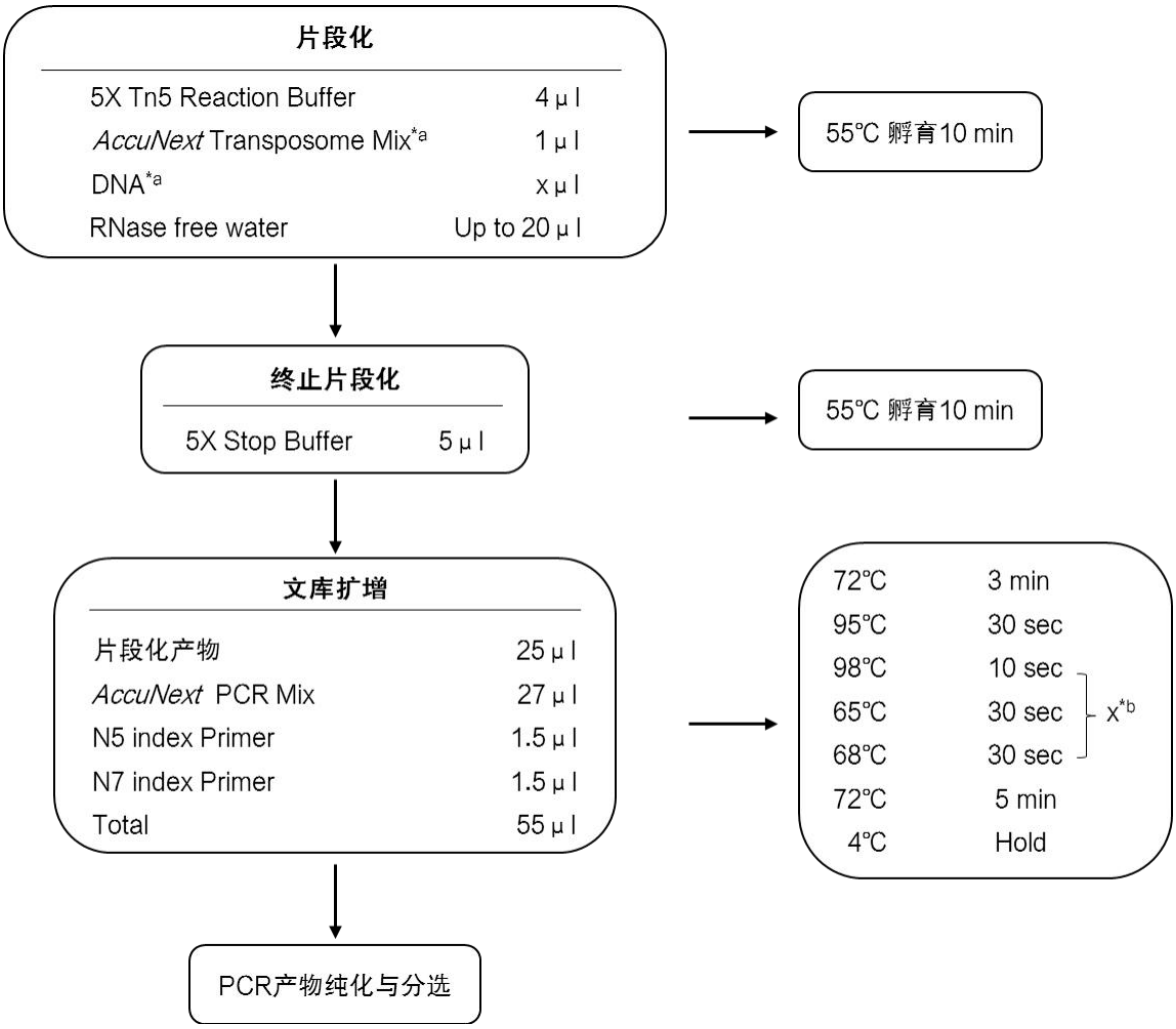


a: The N5/N7 Index Primers can be substituted with the AccuNext Transposome Library Adaptor Primers (Illumina) (Code No. AG12532, AG12533, AG12534).

Figure 2 structure of the final library

5'-AATGATACGGCGACCACCGAGATCTACAC-i5index-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-**NNNNNN**-
CTGTCTCTTATACACATCTCCGAGCCCACGA GAC-i7index-ATCTCGTATGCCGTCTTCTGCTTG-3'

Note: -NNNNNN-=Inserted Target Fragment



5.2 Experimental Precautions

I. Operation Procedure

- Wear a lab coat, disposable mask, and gloves throughout the experiment to prevent DNA contamination or degradation.
- All reagents provided in this kit must be stored and handled in a nuclease-free and nucleic acid-free environment to avoid contamination.
- Use only sterile, nuclease-free consumables. To prevent cross-contamination between samples, it is recommended to use filter tips and change tips between different samples.
- To avoid cross-contamination between experiments, it is recommended to divide the workspace into separate areas:
- Reagent preparation area (preferably with positive airflow), Template addition area, PCR amplification area, Product purification area, Electrophoresis detection area
- Avoid speaking during the experiment. Clean the workbench before and after each experiment using 70% ethanol.
- Carefully open and close sample tubes to prevent splashing or aerosol generation. If samples contact gloves, replace them immediately. If splashes occur on the bench, wipe immediately with water or 70% ethanol.
- For each experiment, include a negative control (no DNA template) to confirm the absence of contamination.

II. DNA Samples

- This product is compatible with four types of purified DNA input amounts (100 ng / 50 ng / 5 ng / 1 ng). Since the AccuNext Transposome Mix is highly sensitive to DNA input quantity, use the amount specified for your kit version. DNA concentration should be measured using fluorescence-based assays (e.g., Qubit, PicoGreen) rather than absorbance-based methods to ensure accuracy and avoid errors that may affect library construction.

- Use high-purity DNA free of proteins, organic solvents, and salts, as these may inhibit enzyme activity and reduce reaction performance.
- When using PCR products for library preparation, ensure that the fragments are longer than 500 bp. Since transposase cannot act on DNA termini, the sequencing coverage at the terminal 50 bp may be reduced. It is therefore recommended to extend both ends of the target region by 50–100 bp during PCR amplification to maintain complete coverage.

III. Library Amplification

- The recommended number of PCR cycles for library amplification is shown below. It is advised to optimize cycle numbers before large-scale experiments. Use the lowest possible cycle number that yields sufficient library quantity to ensure high-quality libraries.

Table 1. Recommended PCR Cycle Numbers

Input DNA Amount	Recommended Cycles	1 μg Library Yield	500 ng Library Yield
100 ng	6–12	10	7
50 ng	6–12	10	8
5 ng	11–15	13	11
1 ng	12–16	16	14

IV. Magnetic Bead Usage for Purification and Size Selection

- Bring magnetic beads to room temperature before use; using cold beads may reduce recovery efficiency and affect size selection.
- Mix the magnetic beads thoroughly before use. Ensure that the DNA sample and beads are fully mixed to achieve optimal binding.
- When removing the supernatant, avoid aspirating any beads, as this may affect library quality.
- Prepare 80% ethanol freshly before each use; old ethanol solutions may decrease DNA recovery efficiency.
- During room temperature drying of magnetic beads, insufficient drying of the 80% ethanol will adversely affect subsequent reactions, while over-drying may cause the beads to crack, resulting in reduced product recovery.

V. Library Quality Control

- Library concentration measurement: It is recommended to use absolute quantification by qPCR for accurate library quantification. Alternatively, fluorescence-based methods such as Qubit or PicoGreen may be used.
- Do not use absorbance-based methods, as they may lead to inaccurate DNA concentration measurements.
- Library size assessment: It is recommended to use the Agilent 2100 Bioanalyzer to evaluate the library fragment size distribution.

5.3 Experiment Preparation

I. Sample Preparation

- Use high-purity DNA with an A260/A280 ratio between 1.8 and 2.0, preferably dissolved in RNase-free water.
- Since the AccuNext Transposome Mix is highly sensitive to DNA concentration, the DNA quantity must be accurately determined using double-stranded DNA fluorescence-based assays such as Qubit or PicoGreen.
- Do not use absorbance-based quantification methods, as they may cause significant deviations in DNA concentration measurement.

II. Reagents & Consumables

- Magnetic bead purification: Use MagSpherix DNA Beads (Accurate Biotech, Code No. AG12546 / AG12547 / AG12548) or an equivalent product such as AMPure XP Reagent (Beckman Coulter Life Sciences, Code No. A63881).
- Library quality control: High Sensitivity DNA Kit (Agilent, Code No. 5067-4626), Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Code No. Q32854) or other equivalent kits.

- Illumina sequencing adapter primers: AccuNext Transposome Library Adaptor Primers (Illumina) (Code No. AG12532 / AG12533 / AG12534)or other equivalent products.
- Other materials: RNase-free water, freshly prepared 80% ethanol, 0.2 mL RNase-free PCR tubes, 1.5 mL microcentrifuge tubes, and low-binding tubes (e.g., Eppendorf, Code No. 022431021 or equivalent).

III. Instruments

- PCR thermal cycler, Qubit 4 Fluorometer, Agilent 2100 Bioanalyzer, micropipettes, vortex mixer, and a bench-top microcentrifuge.

5.4 Experiment Operation Procedure

A. DNA Fragmentation

Thaw all reagents on ice. After thawing, briefly centrifuge, mix thoroughly, and keep on ice.

Note: Mix AccuNext Transposome Mix gently by pipetting up and down; do not vortex. Other reagents can be gently vortexed.

1. Prepare the fragmentation reaction mixture on ice:

Component	Volume (per reaction)
5X Tn5 Reaction Buffer	4 µl
AccuNext Transposome Mix*a	1 µl
DNA*a	X µl
RNase-free water	Up to 20 µl

*a: The volume ratio between AccuNext Transposome Mix and DNA input is as follows:

V100=100 ng, V50=50 ng, V5=5 ng,V1=1 ng

The DNA input amount must be strictly in accordance with the recommended values and should not be adjusted.

It is recommended to dilute DNA with RNase-free water before use.

2. Gently pipette the reaction mixture up and down 10 times, briefly centrifuge, and immediately place the tubes into the PCR thermocycler for fragmentation.

3. Run the following program on the thermocycler (pre-program before use):

Temperature	Time
55 °C	10 min
4 °C	Hold

4. Upon completion, immediately add 5 µl of 5X Stop Buffer, gently mix by pipetting, briefly centrifuge, and place the tubes back into the thermocycler to terminate the fragmentation reaction:

Temperature	Time
55 °C	10 min
4 °C	Hold

⚠ Note: The 5X Stop Buffer must be added immediately after the fragmentation reaction to prevent over-fragmentation, which may result in excessively small library fragments.

5. After the reaction, proceed immediately to Step B. PCR Library Enrichment.

B. PCR Library Enrichment

1. Thaw AccuNext PCR Mix on ice. Gently mix by pipetting up and down, briefly centrifuge, and keep on ice.

2. Prepare the PCR reaction mixture on ice as follows:

Component	Volume (per reaction)
Fragmented DNA product	25 µl
AccuNext PCR Mix	27 µl
N5 index Primer*a	1.5 µl*b
N7 index Primer*a	1.5 µl*b
Total Volume	55 µl

*a: N5/N7 index primers can be substituted with AccuNext Transposome Library Adapter Primers (Illumina) (Code No. AG12532, AG12533, AG12534).

*b: During the fragmentation step, the above three components (AccuNext PCR Mix, N5 index Primer, and N7 index Primer) can be pre-mixed on ice to form a premix. Mix gently by pipetting up and down, and after the fragmentation reaction is completed, add 30 µL of the premix into the fragmentation reaction tube. Mix gently again and briefly centrifuge.

3. Immediately place the reaction tubes in a thermocycler and run the following program (pre-program before use):

Temperature	Time	Cycles
72 °C	3 min	1
95 °C	30 sec	1
98 °C	10 sec	
65 °C	30 sec	X*a
68 °C	30 sec	
72 °C	5 min	1
4 °C	Hold	-

*a: The recommended PCR cycle numbers are listed below. It is advised to optimize cycle numbers experimentally—use the minimum number that yields sufficient library quantity to ensure high-quality libraries.

Input DNA Amount	Recommended Cycles	1 µg Library Yield	500 ng Library Yield
100 ng	6–12	10	7
50 ng	6–12	10	8
5 ng	11–15	13	11
1 ng	12–16	16	14

4. After PCR amplification, place the reaction tubes on ice. The product can be used immediately for purification, or stored at -20°C overnight or -80°C for up to one month.

[To prevent DNA degradation, it is strongly recommended to proceed to the purification step as soon as possible.]

C. Library Purification

PCR products are purified using magnetic beads. Taking MagSpherix DNA Beads (Code No. AG12546, AG12547, AG12548) as an example, a 1.2X bead-to-sample ratio (beads : sample = 1.2 : 1) is recommended for purification.

Note: The optimal bead-to-sample ratio may vary with different bead products. It is recommended to optimize the ratio before use. If using AMPure XP Reagent (Beckman Coulter Life Sciences, Code No. A63881), a 1.0X bead-to-sample ratio is recommended.

I. Experimental Preparation:

- For first-time use, aliquot magnetic beads into 1.5 mL centrifuge tubes and store at 4°C .
- Prepare fresh 80% ethanol before each experiment; approximately 400 µL per sample is required.

- Bring magnetic beads to room temperature (15-25°C) for ~30 minutes before use. Vortex beads thoroughly for ~5 minutes prior to use.

II. Procedure:

1. Add 66 µl of room-temperature magnetic beads to 55 µl PCR product (bead-to-sample ratio 1.2:1). Vortex 5-10 sec or gently mix by pipetting up and down 10 times. Briefly centrifuge.
2. Incubate the bead/PCR mixture at room temperature (15-25°C) for 5 min to allow DNA binding to the beads.
3. Place the PCR tube on a magnetic rack for at least 5 min, until the solution is clear and no beads remain in the supernatant. Carefully remove the supernatant without disturbing the beads.
4. Keep the tube on the magnetic rack, add 200 µl of 80% ethanol (avoid disturbing the beads), incubate at room temperature for 30 sec, and carefully remove the supernatant.
5. Repeat Step 4 once.
6. Keep the tube on the magnetic rack and air-dry the beads with the lid open for 3-5 min, until no ethanol residue remains.
   Note: When beads are completely dry, their surface appears matte. Incomplete drying may reduce DNA elution efficiency and affect downstream reactions. Avoid over-drying, which may cause bead cracking and lower DNA recovery.
7. After drying, remove the tube from the magnetic rack. Add 22 µl RNase-free water to cover the beads, gently mix by pipetting, and incubate at room temperature (15-25°C) for 5 min. If the beads appear cracked or over-dried, extend incubation time as needed.
8. Briefly centrifuge the tube and place it back on the magnetic rack. Separate beads from the solution until clear (~5 min).
9. Carefully transfer 20 µl of supernatant to a new low-binding tube (avoid transferring beads) and store at -20°C.If library fragment sizes are appropriate, proceed directly to Section E. Library Quality Control and sequencing. If fragment sizes require selection, proceed to Section D. Library Size Selection.

D. Library Size Selection

Library size selection is recommended using MagSpherix DNA Beads (Code No. AG12546, AG12547, AG12548). The table below (Table 2) lists recommended bead-to-sample ratios for size selection. If using beads from other sources, ratios may need adjustment.

Table 2. Recommended Bead Ratios for MagSpherix DNA Beads

Average Library Size	300 bp	400 bp	450 bp	500 bp
First Round Beads	90 µl (0.9X)	80 µl (0.8X)	75 µl (0.75X)	70 µl (0.7X)
Second Round Beads	20 µl (0.2X)	20 µl (0.2X)	20 µl (0.2X)	20 µl (0.2X)

Note: Bead volumes are calculated based on 100 µl DNA sample volume. For example, for 0.8X selection: 0.8 x 100 µl = 80 µl

Note: For AMPure XP Reagent (Beckman Coulter Life Sciences, Code No. A63881), the recommended bead ratios are listed in Table 3

Table 3. Recommended Bead Ratios for AMPure XP Reagent

Average Library Size	300 bp	400 bp	500 bp
First Round Beads	80 µl (0.8X)	70 µl (0.7X)	60 µl (0.6X)
Second Round Beads	20 µl (0.2X)	20 µl (0.2X)	20 µl (0.2X)

Experimental Preparation

- For first-time use, aliquot magnetic beads into 1.5 mL centrifuge tubes and store at 4°C.
- Prepare fresh 80% ethanol according to sample number; ~400 µl per sample is required.
- Bring beads to room temperature (15-25°C) for ~30 minutes before use. Vortex beads thoroughly for ~5 min prior to use.

Procedure

1. Adjust the initial DNA sample to 100 µl with RNase-free water before performing dual-round size selection.
2. Add the first-round beads according to Table 2. Vortex or pipette gently 10 times to mix and incubate at room temperature

(15-25°) for 5 min. Bead ratios may be adjusted according to experimental needs.

3. Briefly centrifuge the PCR tube and place it on a magnetic rack. After the solution clears (~5 min), carefully transfer the supernatant to a new tube (leave ~5 µl to avoid aspirating beads).
4. Add the second-round beads according to Table 2.
5. Vortex or pipette gently 10 times to mix, and incubate at room temperature for 5 min.
6. Briefly centrifuge and place the tube on a magnetic rack. After the solution clears (~5 min), carefully remove the supernatant (avoid beads).
7. Keep the tube on the magnetic rack. Add 200 µl 80% ethanol, incubate 30 sec at room temperature, and carefully remove the supernatant.
8. Repeat Step 7 once.
9. Keep the tube on the magnetic rack, open the lid, and air-dry the beads for 3-5 min until no ethanol remains. When beads are dry, the surface appears matte. Incomplete drying may reduce DNA elution efficiency and downstream performance. Avoid over-drying, which may crack beads and reduce recovery.
10. Remove the tube from the magnetic rack, add 22 µl RNase-free water, vortex or pipette gently to mix, and incubate at room temperature for 5 min.
11. Briefly centrifuge and place the tube back on the magnetic rack until the solution is clear (~5 min).
12. Carefully transfer 20 µl of supernatant to a low-binding tube (avoid beads) and store at -20°.If library fragment sizes are appropriate, proceed directly to Section E. Library Quality Control for sequencing. If size selection is required, follow this procedure.

E. Library Quality Control

1. Take 2 µl of purified library and measure concentration using Qubit 4 Fluorometer with Qubit 1X dsDNA HS Assay Kit (Thermo Fisher, Code No. Q33231). Follow the kit instructions.
2. Take 1 µl of purified library and analyze fragment size distribution using Agilent Technologies 2100 Bioanalyzer with Agilent High Sensitivity DNA Kit (Agilent, Code No. 5067-4626). Follow the kit instructions.

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