NGS DNA Library Prep Kit (Mechanical)

N210005

INSTRUCTIONS FOR USE

Ver. EN20250729

CONTENT

Product description

NGS DNA Library Prep Kit (Mechanical) is a next-generation library preparation kit specifically developed for Illumina™ and MGI™ high-throughput sequencing platforms. This product utilizes high-quality enzymatic components, improving upon its predecessor by enhancing the efficiency of DNA fragment end repair and A-tailing, as well as optimizing adapter ligation efficiency. The kit employs a novel high-fidelity enzyme, significantly boosting amplification uniformity and fidelity. It can be applied to various samples including routine plant and animal genomes, microbial genomes, FFPE, cfDNA, ChIP DNA, and more, aiding in obtaining excellent sequencing data.

- Applicable to all DNA samples ranging from 100 pg to 1 μg, including cfDNA and FFPE.
- Offers high conversion efficiency, achieving library conversion rates above 70%.
- Verified across multiple samples for excellent library and sequencing outcomes.
- Undergoes stringent batch performance and stability quality control.

Specifications

Cat.No.	N210005E / N210005S / N210005M
Size	8 T / 24 T / 96 T

Components

Components No.		Name	N210001E	N210001S	N210001M
N210005-A	🟢	Endprep Buffer 2.0	56 μL	168 μL	672 μL
N210005-B	🟡	Endprep Enzyme 2.0	24 μL	72 μL	288 μL
N210005-C	🔴	Ligation Enhancer 2.0	240 μL	720 μL	3×960 μL
N210005-D	🔵	Rapid T4 DNA Ligase 2.0	80 μL	240 μL	2×480 μL
N210005-E	◌	Canace™ Pro Amplification Mix	200 μL	600 μL	3×800 μL

【Note】: When using short adapters(Incomplete adapter), no primer mix is required. However, for Full-length adapters, a primer mix is essential and must be purchased separately. This kit is compatible with both Illumina™ and MGI™ platforms, but requires platform-specific primer mixes:

Cat# N210701 for Illumina™; Cat# N210781 for MGI™.

Storage

This product should be stored at -25~-15°C for 1 year.

Notes

I. About Operation

1. For your safety and health, please wear a lab coat and disposable gloves while handling the reagents.
2. Before use, allow all kit components to thaw at room temperature. After thawing, invert the tubes several times to ensure thorough mixing, briefly centrifuge, and then place on ice until ready for use.
3. When preparing reaction mixtures, it is recommended to pipette up and down or gently vortex to ensure complete mixing. Vigorous shaking or over-vortexing may reduce library yield.

4. To prevent cross-contamination between samples, it is recommended to use filter-barrier pipette tips and change tips when handling different samples.

5. It is recommended to perform all reactions in a thermal cycler with a heated lid. Preheat the thermal cycler to the required reaction temperature before starting the experiment.

6. PCR products are highly susceptible to aerosol contamination due to improper handling, which may affect the accuracy of results. It is strongly recommended to physically separate the area used for PCR setup from the area used for PCR product purification and analysis. Use dedicated pipettes and equipment for each area, and regularly clean workspaces (e.g., wipe surfaces with 0.5% sodium hypochlorite or 10% bleach) to maintain a clean experimental environment.

7. This product is intended for research use only.

II. About DNA Fragmentation

1. This kit is compatible with both mechanically sheared and enzymatically fragmented DNA.

2. The recommended input DNA amount for this kit is 100 pg – 1 µg. High-quality DNA with an A260/A280 ratio of 1.8–2.0 is preferred. Table 1 lists the recommended input DNA amounts for common applications.

Table 1. Recommended Input DNA Amounts for Common Applications

Application	Sample type	Recommended Input DNA
Whole genome sequencing	Complex genome	50 ng-1000 ng
Targeted capture sequencing	Complex genome	10 ng-1000 ng
Whole genome sequencing，Targeted capture sequencing	FFPE DNA	50 ng-1000 ng
Whole genome sequencing，Targeted capture sequencing	cfDNA/ctDNA	≥500 pg
Whole genome sequencing	Microbial genome	≥1 ng
Whole genome sequencing(PCR-free)	High-quality DNA	≥50 ng

[Note]: The recommended Input DNA amounts in the table above are for high-quality DNA. If the quality of the Input DNA is poor or if size selection is required, the amount should be appropriately increased.

3. Input DNA specifically refers to the DNA used in the End Repair&dA-Tailing step.

4. High concentrations of metal ion chelators or other salts carried over during the preparation of Input DNA may affect the efficiency of the End Repair/dA-Tailing reaction. It is recommended to perform magnetic bead purification or size selection after DNA fragmentation. When using mechanical shearing for DNA fragmentation and proceeding directly to library preparation without purification or size selection, dilute the DNA in TE Buffer rather than nuclease-free water.

III. About Adapter Ligation

1. For Illumina™ Sequencing Platforms, Arcegen offers the following adapters:

a. Full-length Adapter Kit for Illumina™, Set 1 ~ Set 2 (Cat#N210706 ~ Cat#N210707), with adapter concentration at 15 µM.

b. DNA Lib Prep 384 CDI Primer for Illumina (Cat#N210731-N210732), with adapter concentration at 15 µM.

c. Stubby UDI Primer Kit for Illumina, PE adapter plus, Set 1 ~ Set 4 (plate format) (Cat#N210761 ~ Cat#N210764), with adapter concentration at 15 µM.

2. For MGI™ High-Throughput Sequencing Platforms, Arcegen offers the following adapters:

a. Full-length Adapter Kit for MGI™, Set 1 ~ Set 2 (Cat#N210703 ~ N210704), with adapter concentration at 10 μM.

b. Unique Dual Barcode Primer Kit for MGI™, Set 1 ~ Set 4 (plate format) (Cat#N210784 ~ Cat#N210787), with adapter concentration at 10 μM.

3. The quality and concentration of adapters directly impact ligation efficiency and library yield. Excessive adapter amounts can lead to higher adapter dimer formation, while insufficient amounts may reduce ligation efficiency and library yield. Dilute the adapters with TE Buffer according to the amount of Input DNA before use.

Table 2 lists the recommended dilution methods for standard and UMI adapters for the Illumina™ sequencing platform using different Input DNA amounts with this kit.

Table 3 lists the recommended dilution methods for standard and UMI adapters for the MGI™ sequencing platform using different Input DNA amounts with this kit.

Table 2. Recommended Concentrations of Standard Adapters for Illumina™ Sequencing Platform Using 100 pg - 1 μg Input DNA

Input DNA	Adapter dilution factor	Concentration
0.1 ng	150-fold dilution	0.1 μM
1 ng	30-fold dilution	0.5 μM
10 ng	7.5-fold dilution	2 μM
100 ng	3-fold dilution	5 μM
1000 ng	1.5-fold dilution	10 μM

Table 3. Recommended Concentrations of Standard and UMI Adapters for MGI™ Sequencing Platform Using 100 pg - 1 μg Input DNA

Input DNA	Adapter dilution factor	Concentration
0.1 ng	100-fold dilution	0.1 μM
1 ng	20-fold dilution	0.5 μM
10 ng	5-fold dilution	2 μM
100 ng	2-fold dilution	5 μM
1000 ng	Undiluted	10 μM

IV. About Bead-Based Clean-Up and Size Selection

1. The DNA fragment size selection step can be performed either before end repair&dA-tailing, after adapter ligation, or after library amplification.

2. When the Input DNA mass is ≥ 50 ng, size selection can be performed after adapter ligation. If the Input DNA mass is < 50 ng, we recommend performing size selection after library amplification.

3. Ligation Enhancer contains a high concentration of PEG, which significantly affects dual-round size selection. Therefore:If performing size selection after adapter ligation, a clean-up step must precede the dual-round size selection.If performing size selection before end repair/dA-tailing or after library amplification, you may proceed directly to dual-round bead-based size selection.

4. Magnetic beads should be brought to room temperature before use; otherwise, this may result in reduced yield and poor size selection performance.

5. Always thoroughly mix the magnetic beads by vortexing or pipetting up and down before each use.

6. When transferring the supernatant, avoid aspirating any beads. Even trace contamination may affect downstream library quality.

7. The 80% ethanol used for bead washing should be freshly prepared prior to use; otherwise, it may reduce recovery efficiency.

8. For size selection, the initial sample volume should be ≥ 100 µL. If less, dilute with nuclease-free water to reach this volume, to minimize pipetting errors.

9. Prior to elution, allow the beads to air-dry at room temperature. Incomplete drying may leave residual ethanol that interferes with downstream reactions, while over-drying may cause bead cracking and lower recovery efficiency. Typically, 3–5 minutes of air drying at room temperature is sufficient.

10. To store purified or size-selected DNA products, elute with TE Buffer. Eluted DNA can be stored at 4 °C for 1–2 weeks, or at –20 °C for up to 1 month.

V. About Library Amplification

1. Whether library amplification is required depends on several factors including the amount of Input DNA, whether full-length adapters are used, and the intended application.Amplification is mandatory when using non-full-length adapters.When using full-length adapters, library amplification is recommended if Input DNA < 200 ng.When Input DNA ≥ 200 ng, or when amplification is not desired, this step may be omitted.

2. The number of PCR cycles during library amplification must be strictly controlled. Too few cycles may result in low yield, while too many cycles may lead to increased bias, duplication rate, chimeric products, and accumulation of amplification-induced mutations.

Table 4. Recommended Number of PCR Cycles for 100 pg – 1 µg Input DNA

Input DNA	Recommended number of PCR cycles for 1 µg Input DNA
1000 ng	2 - 4
500 ng	2 - 4
250 ng	4 - 6
100 ng	5 - 7
50 ng	7 - 9
10 ng	9 - 11
5 ng	10 - 12
1 ng	12 - 15
100 pg	16 - 18

[Note]: ** If using non-full-length adapters, an additional 1–3 PCR cycles are required to generate complete adapter structures. If size selection is performed during library construction, please refer to the higher end of the recommended cycle range for amplification.

VI. About Library Quality Analysis

1. In general, the quality of a constructed library can be evaluated by assessing its fragment size distribution and concentration.

2. Library concentration can be measured using:dsDNA fluorescence dye-based methods, such as Qubit™ or PicoGreen™.qPCR-based absolute quantification methods.

3. The following methods are not recommended for library concentration measurement:

Spectrophotometry-based methods, such as NanoDrop™.

4. We recommend using qPCR-based quantification for library analysis:

Fluorescence dye-based methods (e.g., Qubit™, PicoGreen™) cannot effectively distinguish between:Products with adapter only on one end,products with no adapters on either end,other incomplete double-stranded structures.In contrast, qPCR-based absolute quantification relies on PCR amplification principles and specifically quantifies libraries that have full-length adapters on both ends — i.e., libraries ready for sequencing — thereby excluding non-sequencable libraries from the measurement.

5. For fragment size distribution analysis, it is recommended to use instruments based on capillary electrophoresis or microfluidics, such as the Agilent Bioanalyzer 2100.

Instructions

I. Required Materials (Not Provided)

1. Purification magnetic beads: Cat#N210362, NGS DNA Selection Beads or Cat#A63880, AMPure XP Beads or equivalent products.

2. DNA quality control: Using the Agilent Technologies 2100 Bioanalyzer or an equivalent instrument.

3. DNA Adapters: For detailed adapter information, please refer to Section III ("Adapter Ligation") in the Notes above.

4. DNA Primer Mix: Cat#N210701, DNA Library Prep Primer Mix for Illumina™ or Cat#N210781, DNA Library Prep Primer Mix for MGI™.

5. Other materials: Anhydrous ethanol, nuclease-free ultrapure water, TE Buffer (10 mM Tris-HCl, pH 8.0–8.5; 0.1 mM EDTA), low-binding microcentrifuge tubes, PCR tubes, magnetic rack, PCR thermal cycler, etc.

II. Workflow

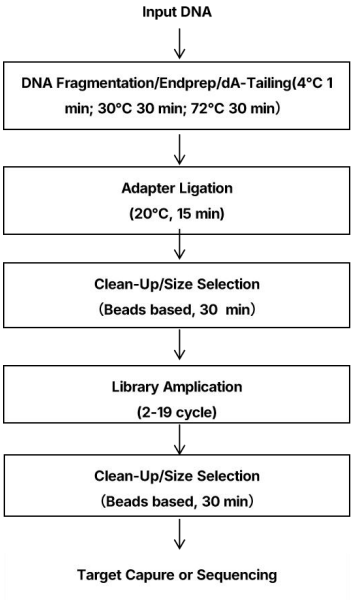

Figure 1. Workflow of NGS DNA Library Prep Kit (Mechanical)

III. Experimental Procedures

3.1 End Repair / dA-Tailing

This step performs end repair and phosphorylation at the 5' ends, as well as dA-tailing at the 3' ends of fragmented input DNA.

1. Thaw the reagents listed in Table 5, mix thoroughly by gentle inversion, and place on ice for immediate use.

2. Prepare the reaction mixture according to the components listed in Table 5 in a sterile PCR tube.

Table 5. Reaction System for End Repair / dA-Tailing

Name	Volume (μL)
Fragmented DNA	x
Endprep Buffer 2.0	7
Endprep Enzyme 2.0	3
ddH_2O	Up to 60

3. Gently pipette to mix, or vortex briefly to ensure thorough mixing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

4. Place the PCR tube into a PCR thermal cycler and run the reaction program as shown in Table 6 to perform end repair and dA-tailing.

Table 6. Reaction Program for End Repair / dA-Tailing

Temperature	Time
Heat Lid 105°C	On
30 °C	30 min
72 °C	30 min
4 °C	Hold

3.2. Adapter Ligation

This step ligates Illumina™ or MGI™ adapters to the ends of the DNA fragments obtained in Step 1).

1. Dilute the adapter to the appropriate concentration according to the recommended adapter usage guidelines in Section 3, based on the amount of input DNA.

2. After thawing the reagents listed in Table 7, mix them thoroughly by gentle inversion and place on ice for immediate use.

3. Prepare the reaction mixture as shown in Table 7 directly in the same PCR tube used in Step 1).

Table 7. Reaction System for Adapter Ligation

Name	Volume (μL)
dA-tailed DNA(1) Step product)	60
Ligation Enhancer 2.0	30*
DNA Adapter	5**
Rapid T4 DNA Ligase 2.0	10
ddH_2O	5
Total	110

[Note]: *The Ligation Enhancer is viscous. Please invert the tube several times or vortex thoroughly to ensure complete mixing, and briefly centrifuge before use to collect the solution at the bottom of the tube.

**The adapter concentrations provided by our company are consistent with those of standard commercial kits: 15 μM for Illumina™ platforms and 10 μM for MGI™ platforms. Please dilute the adapter accordingly based on the instructions in Note III, and adjust the volume with nuclease-free water to a final adapter volume of 5 μL.

4. Gently pipette to mix or vortex briefly to ensure thorough mixing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

5. Place the PCR tube into a thermal cycler and run the reaction program as shown in Table 8 to perform adapter ligation.

Table 8. Reaction Program for Adapter Ligation

Temperature	Time
Heat Lid	Off
20 °C	15 min
4 °C	Hold

3.3. Post Ligation Clean-Up Using Magnetic Beads

This step involves purifying or size-selecting the products from Step 3.2 using magnetic beads. Purification removes unligated adapters or adapter dimers, which are considered non-functional products.

3.3.1 Purification Procedure

1) Preparation: Remove NGS DNA Selection Beads from the refrigerator and allow them to equilibrate to room temperature for at least 30 minutes. Prepare 80% ethanol.

2) Vortex or invert the beads several times to ensure thorough mixing.

3) Add 88 µL of NGS DNA Selection Beads (0.8× ratio, Beads:DNA = 0.8:1) to the Adapter Ligation product. Mix thoroughly by vortexing or gently pipetting up and down. Incubate at room temperature for 5 minutes.

4) Briefly centrifuge the PCR tube and place it on a magnetic rack to separate the beads from the liquid. Once the solution is clear (approximately 5 minutes), carefully remove the supernatant.

5) Keep the PCR tube on the magnetic rack and add 200 µL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove the supernatant.

6) Repeat Step 5 for a total of two washes.

7) With the PCR tube still on the magnetic rack, open the lid and air-dry the beads until cracks just begin to appear (no more than 5 minutes).

8) Remove the PCR tube from the magnetic rack and proceed with elution:

a. If no fragment selection is required, add 21 µL of ddH_2O directly. Mix thoroughly by vortexing or gently pipetting up and down. Incubate at room temperature for 5 minutes.

[Note]: For long-term storage of purified products, use TE Buffer for elution.

b. Briefly centrifuge the PCR tube and place it back on the magnetic rack. Once the solution is clear (approximately 5 minutes), carefully transfer 20 µL of the supernatant to a new PCR tube without disturbing the beads.

c. If double-round size selection is needed, add 102 µL of ddH_2O and perform size selection with an appropriate amount of beads.

[Note]: If double-round size selection is needed, add 102 µL of ddH_2O and perform size selection with an appropriate amount of beads.

3.3.2 Double-Round Size Selection Procedure

1) Preparation: Remove NGS DNA Selection Beads from the refrigerator and allow them to equilibrate to room temperature for about 30 minutes. Prepare 80% ethanol.

2) Vortex or invert the beads several times to ensure thorough mixing.

3) According to the desired DNA fragment size, refer to Table 9 for the recommended bead volume to add to the 100 µL of DNA supernatant. Mix thoroughly by vortexing or pipetting up and down 10 times.

Table 9. Recommended Bead Ratios for Library Size Selection

DNA Library Size (Insert Fragment Length)	150 ~ 250 bp	200 ~ 300 bp	300 ~ 400 bp	400 ~ 500 bp
DNA library size	250 ~ 350 bp	350 ~ 450 bp	450 ~ 550 bp	550 ~ 650 bp
First-Round Bead Volume Ratio (Beads:DNA)	0.80×	0.70×	0.60×	0.55×
Second-Round Bead Volume Ratio (Beads:DNA)	0.20×	0.20×	0.20×	0.15×

[Note]: In the table, "×" represents the sample DNA volume. For example, if the library insert size is 250 bp and the sample DNA volume is 100 µL, the first-round bead volume would be 0.70 × 100 µL = 70 µL; the second-round bead volume would be 0.20 × 100 µL = 20 µL. The recommended ratios in the table are for Adapter Ligated Insert DNA (Post Ligation). If users perform size selection before adapter ligation, please refer to the recommended ratios in the NGS DNA Selection Beads (Cat#N210362) manual.

4) Incubate at room temperature for 5 minutes.

5) Briefly centrifuge the PCR tube and place it on a magnetic rack. Once the solution is clear (approximately 5 minutes), carefully transfer the supernatant to a clean microcentrifuge tube.

6) Refer to Table 9 and add the second-round size selection beads to the supernatant.

7) Mix thoroughly by vortexing or pipetting up and down 10 times. Incubate at room temperature for 5 minutes.

8) Briefly centrifuge the PCR tube and place it back on the magnetic rack. Once the solution is clear (approximately 5 minutes), carefully remove the supernatant.

9) Keep the PCR tube on the magnetic rack and add 200 µL of freshly prepared 80% ethanol to wash the beads. Incubate at room temperature for 30 seconds, then carefully remove the supernatant.

10) Repeat step 9).

11) With the PCR tube still on the magnetic rack, open the lid and air-dry the beads until cracks just begin to appear (about 5 minutes).

12) Remove the PCR tube from the magnetic rack and add an appropriate volume of 21 µL ddH_2O. Mix thoroughly by vortexing or gently pipetting up and down. Incubate at room temperature for 5 minutes.

13) Briefly centrifuge the PCR tube and place it back on the magnetic rack to separate the beads from the liquid. Once the solution is clear (approximately 5 minutes), carefully transfer 20 µL of the supernatant to a clean tube.

3.4. Library Amplification

This step involves PCR amplification to enrich the purified or size-selected adapter-ligated products.

1) After thawing the reagents listed in Table 10, mix them thoroughly by inversion and keep them on ice for immediate use.

2) Prepare the reaction mixture as shown in Table 10 in a sterile PCR tube.

Table 10. PCR Amplification Reaction System

Name	Volume (μL)
Adapter Ligated DNA(Step3 product)	20
Canace™ Pro Amplification Mix	25
Primer mix**	5*
Total	50

[Note]:*Primer Mix: Adapters and Primer Mixes are selected according to the corresponding sequencing platform.

**If using Illumina completed adapters, use the Primer Mix from the DNA Library Prep Primer Mix for Illumina (Cat#N210701) for amplification.

If using MGI completed adapters, use the Primer Mix from the DNA Library Prep Primer Mix for MGI (Cat#N210781) for amplification.

If using incomplete adapters from either platform, refer to the respective adapter kit manual and use the Index Primer provided in the kit for amplification.

3) Gently pipette to mix or vortex briefly to ensure thorough mixing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

4) Gently pipette to mix or vortex briefly to ensure thorough mixing, then briefly centrifuge to collect the reaction mixture at the bottom of the tube.

Table 11. PCR Amplification Reaction Program

Temperature	Time	Cycle number
98 °C	45 sec	1
98 °C	15 sec	Refer to Table 4 in the notes.
60 °C	30 sec	
72 °C	30 sec	
72 °C	1 min	1
4 °C	Hold	-

3.5. Post Amplification Clean-Up / Size Selection

Follow the same procedure as described for post-ligation clean-up. Use NGS DNA Selection Beads (1× ratio, Beads : DNA = 1:1) to purify the amplified library products.

3.6. Library Quality Analysis

Typically, the quality of the constructed library can be assessed through concentration measurement and size distribution analysis.