EpiNext™ DNA Library Preparation Kit (Illumina)

Base Catalog # P-1051

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ DNA Library Preparation Kit (Illumina) is suitable for preparing a DNA library for next generation sequencing applications using an Illumina sequencer, which includes genomic DNA-seq, ChIP-seq, MeDIP/hMeDIP-seq, bisulfite-seq, and targeted re-sequencing. The optimized protocol and components of the kit allow both non-barcoded (singleplexed) and barcoded (multiplexed) DNA libraries to be constructed quickly with reduced bias.

Starting Material and Input Amount: Starting materials can include fragmented dsDNA isolated from various tissue or cell samples, dsDNA enriched from ChIP reaction, MeDIP/hMeDIP reaction or exon capture. DNA should be relatively free of RNA since large fractions of RNA will impair end repair and dA tailing, resulting in reduced ligation capabilities. Input amount of DNA can be from 5 ng to 1 ug. For optimal preparation, the input amount should be 100 ng to 200 ng. For amplification-free, 500 ng or more is needed.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	12 reactions Cat. #P-1051-12	24 reactions Cat. #P-1051-24	Storage Upon Receipt
10X End Repair Buffer*	40 µl	80 µl	-20°C
End Repair Enzyme Mix*	25 µl	50 µl	-20°C
10X dA-Tailing Buffer*	40 µl	80 µl	-20°C
Klenow Fragment (3'-5' exo$^-$)*	15 µl	30 µl	-20°C
2X Ligation Buffer*	250 µl	500 µl	-20°C
T4 DNA Ligase*	15 µl	30 µl	-20°C
Adaptors (50 µM)*	15 µl	30 µl	-20°C
MQ Binding Beads*	1.6 ml	3.2 ml	4°C
2X HiFi PCR Master Mix*	160 µl	320 µl	-20°C
Primer U (10 µM)*	15 µl	30 µl	-20°C
Primer I (10 µM)*	15 µl	30 µl	-20°C
Elution Buffer*	1000 µl	2000 µl	-20°C

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: Store the following components at -20°C immediately: **10X End Repair Buffer**, **End Repair Enzyme Mix**,**10X dA-Tailing Buffer**, **Klenow Fragment (3'-5' exo$^-$)**, **2X Ligation Buffer**, **T4 DNA Ligase**, **Adaptors**, **2X HiFi PCR Master Mix**, **Primer U**, **Primer I**, and **Elution Buffer**. Store the following components at 4°C: **MQ Binding Beads**. Store all other components at room temperature.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Sonicator or enzymes for DNA fragmentation
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of DNA library
- ☐ Thermocycler
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Magnetic stand (96-well format)
- ☐ Pipettes and pipette tips
- ☐ PCR tubes or plates
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 100% ethanol
- ☐ Distilled water
- ☐ DNA sample

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of EpiNext™ DNA Library Preparation Kit (Illumina) is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiNext™ DNA Library Preparation Kit (Illumina) is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiNext™ DNA Library Preparation Kit (Illumina) and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

DNA library preparation is a critical step for next generation sequencing (NGS). For generating accurate sequencing data for NGS, the prepared library DNA should be sufficient in yield and of high quality. Also, as NGS technology is continuously improving, DNA library preparation is required to be optimized accordingly. For example, most of the currently used methods are time-consuming, expensive and inconvenient. Some of the methods are relatively quick by combining end repair and dA tailing or even ligation in one-step. However, these methods have been shown to generate significant G tailing or form concatemers at the ligation step or to have high insertion bias. These side reactions eventually result in the prepared DNA library being less efficient and inaccurate. An ideal DNA library preparation method should be balanced in speed, convenience, small sample-suitability, cost-effectiveness and accuracy. To address this issue, EpigenTek offers the EpiNext™ DNA Library Preparation Kit (Illumina). This kit has the following features:

- Fast and streamlined procedure: The procedure from fragmented DNA to size selection is less than 2 h 30 min. Only one clean-up between each step, thereby saving time and preventing handling errors, as well as loss of valuable samples. Gel-free size selection further reduces the preparation time.
- The most convenient for use: The kit contains all required components for each step of DNA library preparation, which are sufficient for end repair, dA tailing, ligation, clean-up, size selection and library amplification, thereby allowing the library preparation to be convenient with reliable and consistent results.
- Minimized bias: Ultra HiFi amplification and an optional PCR-free step enable the user to achieve reproducibly high yields of DNA library with minimal sequence bias and low error rates.
- Flexibility: Can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation. Uses various dsDNA including fragmented dsDNA isolated from various tissue or cell samples, dsDNA enriched from ChIP reactions, MeDIP/hMeDIP reactions or exon capture.

Broad range of input DNA from 5 ng to 1 µg. PCR-free library preparation can be performed with use of 500 ng or more input DNA.

PRINCIPLE & PROCEDURE

The EpiNext™ DNA Library Preparation Kit (Illumina) contains all reagents required at each step for carrying out successful DNA library preparation. In the library preparation, DNA is first fragmented to the appropriate size (about 300 bp peak size). The end repair of the DNA fragments is performed and an A-overhang is added at the 3'-end of each strand. Adaptors are then ligated to both ends of the end repaired/dA tailed DNA fragments for amplification and sequencing. Fragments are then size selected and purified with MQ beads, which allows quick and precise size selection of DNA. Size-selected DNA fragments are then amplified with a high-fidelity PCR Mix which ensures maximum yields from minimum amounts of starting material and provides highly accurate amplification of library DNA with low error rates and minimum bias.

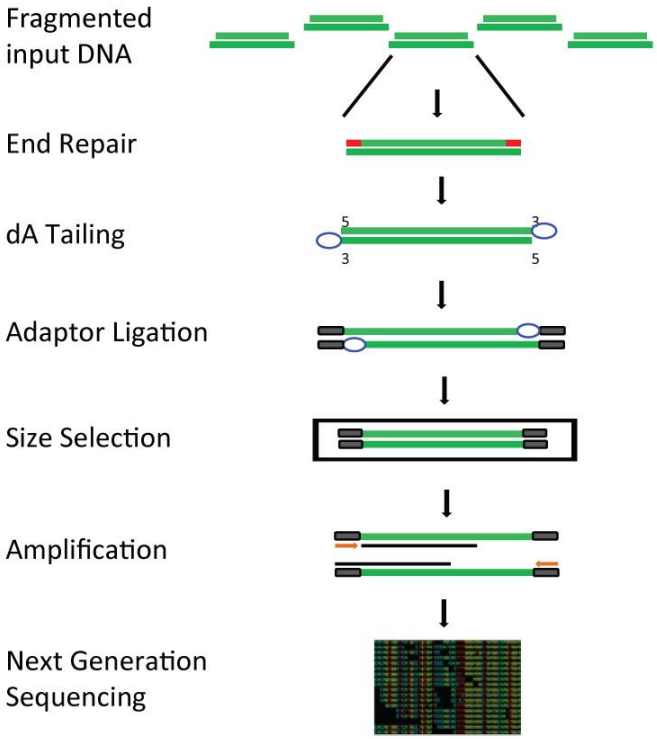

Fig 1. Workflow of the EpiNext™ DNA Library Preparation Kit (Illumina)

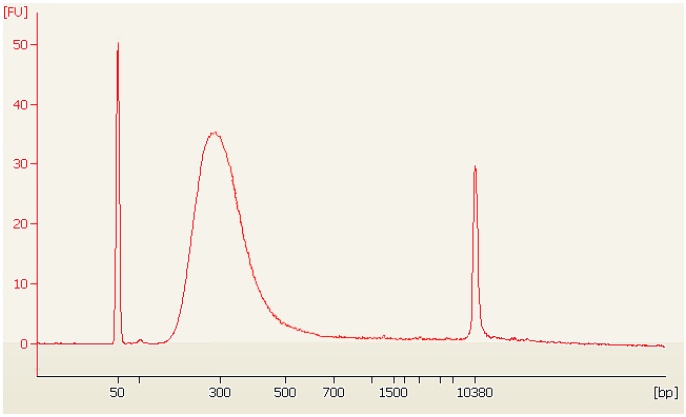

Fig2. Size distribution of library fragments. Human placenta DNA was sheared to 210 bps in peak size and 20 ng of sheared DNA was used for DNA library preparation using EpiNext™ DNA Library Preparation Kit (Illumina).

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Fragmented dsDNA that is isolated from various tissues or cell samples: 5 ng-1 µg, optimal 100-200 ng per preparation. For amplification-free, 500 ng or more of DNA is needed.
dsDNA enriched from ChIP reactions, MeDIP/hMeDIP reactions or exon capture: 5 ng-500 ng.
DNA should be high quality relatively free of RNA. RNAse I can be used to remove RNA and DNA should be eluted in DNase/RNase-free water.

DNA Fragmentation

dsDNA enriched from ChIP reactions, MeDIP/hMeDIP reactions or exon capture should be fragmented already. DNA isolated from various tissue or cell samples can be fragmented using one of the following methods. For the best results we highly recommend to use waterbath-based sonication device. The peak size of fragmented DNA should be compatible with the read length of the Illumina sequencing platform to be used. In general, the peak size of fragments should be about 300 bps.

Waterbath Sonication:

EpigenTek's EpiSonic 1100 (EpigenTek Cat No. EQC-1100): For a target peak size of 300 bps, use 20 µl of DNA solution (50-500 ng) per 0.2 ml tube or per PCR plate well. Shear 60 cycles under cooling conditions, 30 seconds ON, 15 seconds OFF, each at 120-130 watts. For more detailed information of use, please see the "DNA Shearing Protocol" for EpiSonic 1100.

If using other waterbath sonicators please follow the supplier's recommended instructions.
Enzymatic Shearing:

The DNA can also be sheared using various enzyme-based methods. Optimization of the shearing conditions, for example enzyme concentration and incubation time, is needed in order to use enzyme-based methods.

1. DNA End Repairing

a. Prepare end repair reaction in a 0.2 ml PCR tube according to Table 1:

Table 1. End Repair Reaction

Component	Volume
Fragmented DNA (5-500 ng)*	2-10 µl
10X End Repair Buffer	2 µl
End Repair Enzyme Mix	1 µl
Distilled Water	7-15 µl
Total volume	**20 µl**

***Note**: *The optimized amount of fragmented DNA is 100-200 ng*

b. Mix and incubate for 30 min at 20°C in a thermocycler (without a heated lid).

2. Clean-up of End Repaired DNA

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 36 µl of resuspended beads to the PCR tube of end repair reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 10 minutes at room temperature to allow DNA to bind to beads.

d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*

e. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

f. Repeat Step 2e for a total of two washes.

g. Open the cap of the PCR tube and air dry beads for 10 minutes while the tube is on the magnetic stand.

h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.

i. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.

j. Transfer 11-12 ul of the supernatant to a new 0.2 ml PCR tube for the dA-tailing reaction.

3. DNA dA-Tailing

a. Prepare the reaction mix for dA tailing according to Table 2. Add the following reagents to 0.2 ml PCR tube containing end repaired DNA from Step 1.

Table 2. dA-Tailing Reaction

Component	Volume
End repaired DNA (from step 2)	11-12 µl
10X dA-Tailing Buffer	1.5 µl
Klenow Fragment (3'-5' exo$^-$)	1 µl
Distilled Water	0.5-1.5 µl
Total Volume	**15 µl**

b. Mix & incubate for 30 min at 37°C followed by 10 min at 75°C in a thermocycler without heated lid.

4. Adaptor Ligation

a. Prepare a reaction mix for adaptor ligation according to Table 3. Add the following reagents to a 0.2 ml PCR tube containing end repaired/dA-Tailing DNA from Step 3.

Table 3. Adaptor Ligation

Component	Volume
End repaired/dA-Tailing DNA (from step 3)	15 µl
2X Ligation Buffer	17 µl
T4 DNA Ligase	1 µl
Adaptors	1 µl
Total volume	**34 µl**

b. Mix and incubate for 10 min at 25°C in a thermocycler (without heated lid).

Note: *(1) The pre-annealed adapters included in the kit are suitable for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation and are fully compatible with*

Illumina platforms, such as MiSeq® or HiSeq™ sequencers. (2) If using adaptors from other suppliers (both single-end and barcode adaptors), make sure they are compatible with Illumina platforms and add the correct amount (final concentration 1.5-2 µM, or according to the supplier's instruction).

5. Size Selection/Clean-up

5.1. Size Selection of Ligated DNA

***Note**: If the starting DNA amount is less than 50 ng, the size selection is not recommended and alternatively, clean-up of ligated DNA can be performed prior to PCR amplification according to section 5.2 of the user guide.*

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 14 µl of resuspended **MQ Binding Beads** to the tube of ligation reaction. Mix well by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature.
d. Put the tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully transfer the supernatant containing DNA to a new tube *(Caution: Do not discard the supernatant.)* Discard the beads that contain the unwanted large fragments.
e. Add 10 µl resuspended beads to the supernatant, mix well and incubate for 5 minutes at room temperature.
f. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*
g. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
h. Repeat Step 5.1g for a total of two washes.
i. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
j. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
k. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
l. Transfer 11 µl of supernatant to a new 0.2 ml PCR tube for PCR amplification.

5.2. Clean-up of Ligated DNA (Optional)

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 34 µl of resuspended beads to the PCR tube of ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*
e. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step 5.2e two times for a total of three washes.
g. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
i. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.

j. Transfer 11 µl of supernatant to a new 0.2 ml PCR tube for PCR amplification.

If the library is prepared using 500 ng or more input DNA, the library DNA can be directly used for sequencing application after size selection. Otherwise, go to Step 6 for PCR amplification.

6. Library Amplification

a. Prepare the PCR Reactions

Thaw all reaction components including **2X HiFi PCR Master Mix**, primers and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to –20˚C immediately following use. Add components into each PCR tube/well according to the following table:

Component	Size (µl)
2X HiFi PCR Master Mix	12.5 µl
Primer U	1 µl
Primer I (or barcode)	1 µl
Adaptor Ligated DNA	10.5 µl
Total Volume	**25 µl**

Important Note: *Use of **Primer I** included in the kit will generate a singleplexed library. For multiplexed library preparation, replace **Primer I** with one of the12 different barcodes (indexes) contained in the EpiNext™ NGS Barcode (Index) Set-12 (Cat. No. P-1060). You can also add user-defined barcodes (Illumina compatible) instead of **Primer I**.*

b. Program the PCR Reactions

Place the reaction plate in the instrument and set the PCR conditions as follow:

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	10 sec 15 sec 20 sec	Variable*
Final Extension	72°C	1 min	1

** PCR cycles may vary depending on the input DNA amount. In general, use 8 PCR cycles for 500 ng, 12 cycles for 50 ng, and 16 cycles for 5 ng DNA input. Further optimization of PCR cycle number may be required.*

7. Clean-up of Amplified Library DNA

a. Resuspend **MQ Binding Beads** by vortex.
b. Add 25 µl of resuspended beads to the PCR tube of amplification reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain DNA.)*

e. Keep the PCR tube in the magnetic stand and add 200 µl of freshly prepared *80% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step 7e two times for total of three washes.
g. Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 22 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
i. Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear.
j. Transfer 20 µl of supernatant to a new 0.2 ml PCR tube.

Quality of the prepared library can be assessed using an Agilent® Bioanalyzer® or a comparable method. Library fragments should have the correct size distribution (ex: 300 bps at peak size) without adaptors or adaptor-dimers.

To check the size distribution, dilute library 5-fold with water and apply it to an Agilent® high sensitivity chip. If there is presence of <150 bp adaptor dimers or of larger fragments than expected, they should be removed. To remove fragments below 150 bps use 0.8X **MQ Binding Beads Beads** (ex: add 16 µl of **MQ Binding Beads** to 20 µl of sample) according to sub-steps a through i of Step 5.2 “Clean-up of Ligated DNA”. To remove fragments above 500 bps follow sub-steps a through l of Step 5.1 “Size Selection of Ligated DNA”.

The prepared DNA library can be quantified using various DNA library quantification methods and can be stored at -20˚C until ready to use for sequencing.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of library	Insufficient amount of starting DNA.	To obtain the best results, the amount of input DNA should be 100-200 ng. For library directly used for sequencing without amplification, 500 ng or more is needed.
	Insufficient purity of starting DNA.	Ensure that RNA is removed by RNase treatment before starting library preparation protocol.
	Improper reaction conditions at each reaction step.	Check if the reagents are properly added and incubation temperature and time are correct at each reaction step including End Repair, dA Tailing, Adaptor Ligation, Size Selection and Amplification.
	Improper kit storage.	Ensure that the kit has not exceeded the expiration date. Standard shelf-life, when stored properly, is 6 months from date of receipt.
Unexpected peak size of Agilent® Bioanalyzer® trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected.	Improper ratio of MQ beads to DNA volume during size selection.	Check if the correct volume of MQ beads is added to the DNA solution accordingly. Proper ratios should remove the fragments of unexpected peak size. See Step 7 for more details.
	Insufficient ligation.	Too much or too little input DNA may cause insufficient ligation, which can shift the peak size of the fragment population to be shorter or larger than expected. Make sure that the ligation reaction is properly processed using the proper amount of input DNA.

	Over-amplification of library.	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use proper PCR cycles to avoid this problem.

RELATED PRODUCTS

DNA Isolation and Cleanup

P-1003 FitAmp™ General Tissue Section DNA Isolation Kit
P-1004 FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006 DNA Concentrator Kit
P-1009 FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017 FitAmp™ Urine DNA Isolation Kit
P-1018 FitAmp™ Blood and Cultured Cell DNA Extraction Kit

DNA Enrichment Reaction

P-1015 Methylamp™ Methylated DNA Capture (MeDip) Kit
P-1038 EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit
P-1052 EpiQuik™ MeDIP Ultra Kit
P-2002 EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
P-2003 EpiQuik™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
P-2014 EpiQuik™ Plant ChIP Kit
P-2025 ChromaFlash™ One-Step ChIP Kit
P-2026 ChromaFlash™ One-Step Magnetic ChIP kit
P-2027 ChromaFlash™ High-Sensitivity ChIP Kit

DNA Bisulfite Conversion

P-1001 Methylamp™ DNA Modification Kit
P-1026 BisulFlash™ DNA Modification Kit

DNA Library Prep

P-1053 EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina)
P-1055 EpiNext™ Post-Bisulfite DNA Library Preparation Kit (Illumina)
P-1056 EpiNext™ Bisulfite-Seq High Sensitivity Kit (Illumina)

NGS Barcode

P-1060 EpiNext™ NGS Barcode (Index) Set-12

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