ab185903 – DNA Library Preparation Kit (For Illumina®)

Instructions for Use

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

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

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 Abcam offers the DNA Library Preparation Kit (For Illumina®).

This kit has the following features:

- Fast and streamlined procedure: The procedure from fragmented DNA to size selection is less than 2 hours 30 minutes. 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.

The DNA Library Preparation Kit (For 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.

The DNA Library Preparation Kit (For 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.

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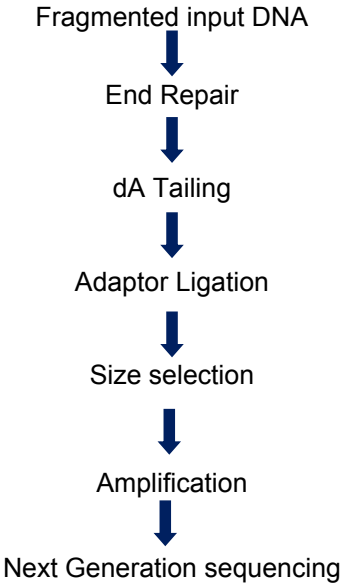
2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

5. MATERIALS SUPPLIED

Item	24 Tests	48 Tests	Storage Condition (Before Preparation)
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

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Sonicator or enzymes for DNA fragmentation
- 1.5 mL microcentrifuge tubes
- Vortex mixer
- Thermocycler with 48 or 96-well block
- Centrifuge (up to 14,000 rpm)
- PCR tubes or Plates
- 100% Ethanol
- Distilled Water
- DNA sample
- DNA analyzer or comparable method to assess the quality of DNA library
- Magnetic Stand(96-well format)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. SAMPLE PREPARATION

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.

9.1 Starting Materials

Fragmented dsDNA that is isolated from various tissues or cell samples: 5 ng - 1 µg, optimal 100 ng - 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.

9.2 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 a water bath-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 bp.

9.2.1 Water Bath Sonication - Please follow the supplier's recommended instructions.

9.2.2 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.

10. ASSAY PROCEDURE

10.1 DNA End Repairing

10.1.1 Prepare end repair reaction in a 0.2 mL PCR tube according to Table below:

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

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

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

10.2 Clean-up of End Repaired DNA

10.2.1 Resuspend MQ Binding Beads by vortex.

10.2.2 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.

10.2.3 Incubate for 10 minutes at room temperature to allow DNA to bind to beads.

10.2.4 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.)

10.2.5 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 minute, and then carefully remove and discard the ethanol.

10.2.6 Repeat previous Step for a total of two washes.

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

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

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

10.2.10 Transfer 11-12 µL of the supernatant to a new 0.2 mL PCR tube for the dA-tailing reaction.

10.3 DNA dA Tailing

10.3.1 Prepare the reaction mix for dA tailing according to Table below. Add the following reagents to 0.2 mL PCR tube containing end repaired DNA from Step 10.2.

Component	Volume
End repaired DNA (from 10.2)	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

10.3.2 Mix and incubate for 30 minutes at 37°C followed by 10 minutes at 75°C in a thermocycler (without heated lid).

10.4 Adaptor Ligation

10.4.1 Prepare the reaction mix for adaptor tailing according to Table below. Add the following reagents to 0.2 mL PCR tube containing end repaired/dA-Tailing from Step 10.3.

Component	Volume
End repaired DNA (from 10.3)	15 µL
2X Ligation Buffer	17 µL
T4 DNA Ligase	1 µL
Adaptors	1 µL
Total volume	34 µL

10.4.2 Mix and incubate for 10 minutes 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.*

(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).

10.5 Size Selection/Clean-up

10.5.1 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 10.5.2 of the user guide.*

10.5.1.1 Resuspend MQ Binding Beads by vortex.

10.5.1.2 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.

10.5.1.3 Incubate for 5 minutes at room temperature.

10.5.1.4 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.

10.5.1.5 Add 10 µL resuspended beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

10.5.1.6 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.).

10.5.1.7 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 minute, and then carefully remove and discard the ethanol.

10.5.1.8 Repeat previous Step for a total of two washes.

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

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

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

10.5.1.12 Transfer 11 µL of supernatant to a new 0.2 mL PCR tube for PCR amplification.

10.5.2 Clean-Up of Ligated DNA (Optional)

10.5.2.1 Resuspend MQ Binding Beads by vortex.

10.5.2.2 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.

10.5.2.3 Incubate for 5 minutes at room temperature to allow DNA to bind to beads.

10.5.2.4 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.)

10.5.2.5 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 minute, and then carefully remove and discard the ethanol.

10.5.2.6 Repeat previous Step two times for a total of three washes.

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

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

10.5.2.9 Capture the beads by placing the tube in the magnetic stand for 4 minutes or until the solution is completely clear. 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 10.6 for PCR amplification

10.6 Library Amplification

10.6.1 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	Volume/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 another barcoded. You can also add user-defined barcodes (Illumina® compatible) instead of Primer I.*

10.6.2 Program the Thermocycler.

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

Cycle Step	Temperature	Time	Negative control
Activation	98°C	30 seconds	1
Cycling	98°C	10 seconds	Variable*
	55°C	15 seconds	
	72°C	20 seconds	
Final Extension	72°C	60 seconds	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.

10.7 **Clean-up of Amplified Library DNA**

10.7.1 Resuspend MQ Binding Beads by vortex.

10.7.2 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.

10.7.3 Incubate for 5 minutes at room temperature to allow DNA to bind to beads.

10.7.4 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.)

10.7.5 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 minute, and then carefully remove and discard the ethanol.

10.7.6 Repeat previous Step two times for total of three washes.

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

10.7.8 Resuspend the beads in 22 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

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

10.7.10 Transfer 20 µL of supernatant to a new 0.2 mL PCR tube.

Quality of the prepared library can be assessed using a DNA 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 a DNA analyzer 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 (ex: add 16 µL of MQ Binding Beads to 20 µL of sample) according to the first 9 Steps of Step 10.5.2

“Clean-up of Ligated DNA”. To remove fragments above 500 bps follow the first 9 Steps of Step 10.5.1 “Size Selection of Ligated DNA”.

The prepared DNA library can be quantified using various DNA library quantification methods.

11. ANALYSIS

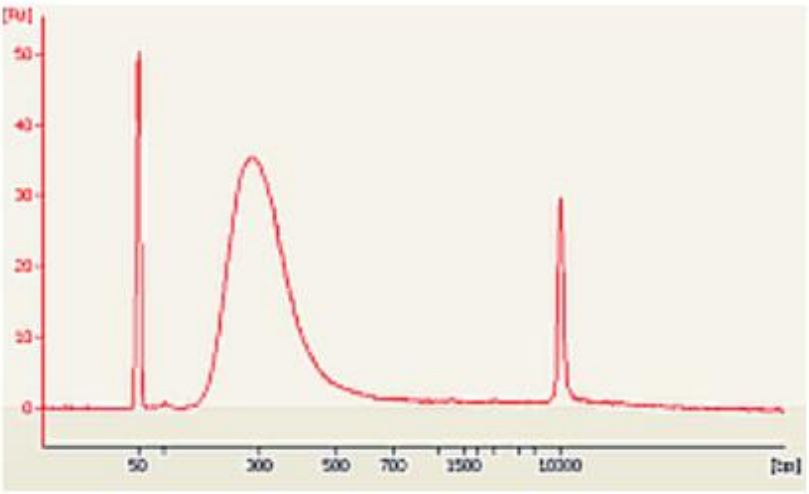


Figure 1. Human placenta DNA was sheared to 210 bp peak size and 20 ng of sheared DNA was used for DNA library preparation.

12. TROUBLESHOOTING

Problem	Cause	Solution
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.

Problem	Cause	Solution
Unexpected peak size of DNA 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 10.7 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.

13. NOTES

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