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ab185905 –
High Sensitivity DNA Library Preparation Kit (For Illumina®)

Instructions for Use

For the preparation of a DNA library using sub-nanogram amounts of DNA input for next generation sequencing applications using an Illumina® sequencer

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 in 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, inconvenient, and specifically need large amounts of DNA. These reactions result in a DNA library preparation which cannot be used for biological samples with limited amounts of starting material such as tumor biopsy, early embryos, embryonic tissues and circulating DNA. In addition, the amount of DNA enriched by ChIP or MeDIP/hmeDIP is often at low or sub-nanogram levels which causes insufficient DNA library yields. To address this issue, Abcam offers the High-Sensitive DNA Library Preparation Kit (For Illumina®).

This kit has the following features:

- High sensitivity and flexibility: - Can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation. The amount of input DNA can be as low as 0.2 ng with a range from 0.2 to 100 ng. Various dsDNA can be used, which includes limited amounts of fragmented dsDNA isolated from various tissue or cell samples, dsDNA enriched from ChIP reactions, and dsDNA enriched from MeDIP/hMeDIP reactions or exon capture
- Fast and streamlined procedure: - The procedure from fragmented DNA to size selection is less than 1 hour and 30 minutes. No clean-up is required between each step and all reactions take place in the same tube, thereby saving time and preventing handling errors as well as loss of valuable samples. Gel-free size selection further reduces the preparation time
- Highly convenient: - The kit contains all required components for each step of DNA library preparation, which are sufficient for end polishing, ligation, clean-up, size selection, and library amplification,

thereby allowing the library preparation to be streamlined with the most reliable and consistent results
- Minimized bias: Ultra HiFi amplification enables to reproducibly achieve high yields of DNA library with minimal sequence bias and low error rates.

The High Sensitivity DNA Library Preparation Kit (For Illumina®) is suitable for preparing a DNA library using sub-nanogram amounts of DNA input for next generation sequencing applications using an Illumina® sequencer. These applications include genomic DNA-seq, ChIP-seq, MeDIP/hMeDIP-seq, classical 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.

ab185905 contains all reagents required at each step of workflow for carrying out successful DNA library preparation. In the library preparation, DNA is first fragmented to appropriate size (about 300 bps in peak size). The end repair/dA tailing (end polishing) of the DNA fragments are performed simultaneously. Adaptors are then ligated to both ends of the polished DNA fragments for amplification and sequencing. Ligated fragments are size selected and purified with MQ beads, which allows quick and precise size selection of DNA. Size-selected DNA fragments are amplified with high-fidelity PCR Mix that 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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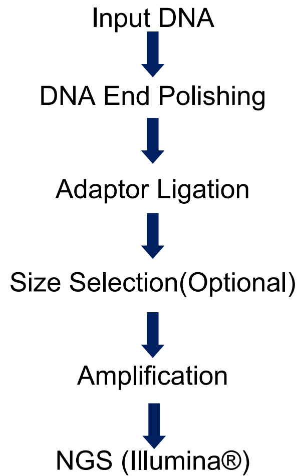
2. ASSAY SUMMARY



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 Polishing Buffer	30 µL	60 µL	–20°C
End Polishing Enzyme Mix	13 µL	26 µL	–20°C
End Polishing Enhancer	13 µL	26 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	1 mL	2 mL	–20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Vortex Mixer
- Sonicator or enzymes for DNA fragmentation
- 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
- 80% Ethanol
- Distilled water
- DNA Sample

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. REAGENT PREPARATION

All reagents are supplied ready to use.

10. SAMPLE PREPARATION

10.1 **Starting Materials:** Fragmented dsDNA that is isolated from various tissues or cell samples: 0.2 ng-100 ng, optimized 10-50 ng per preparation.
dsDNA enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture: 0.2 ng-100 ng. DNA should be of high quality and relatively free of RNA. RNAse I can be used to remove RNA and DNA should be eluted in DNase/RNase-free water.

10.2 **DNA Fragmentation:** dsDNA enriched from a ChIP reaction, MeDIP/hMeDIP reaction or exon capture should already be fragmented. DNA isolated from various tissue or cell samples can be fragmented using one of the following methods. For the best results we highly recommend using a 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 200-300 bps.

10.3 **Waterbath Sonication:** For target peak size 200 bps, use 10 µL of DNA solution (standard amount 10-50 ng) per 0.2 mL PCR tube. Shear 40 cycles under cooling condition, 45 seconds On, 15 seconds Off, each at 110-120 watts. For more specific information please follow the waterbath sonicator supplier's instructions.

10.4 **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.

11. ASSAY PROCEDURE

11.1 DNA End Polishing

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

Component	Sample (µL)
Fragmented DNA	10 (10-50 ng)
10X End Polishing Buffer	1.5
End Polishing Enzyme Mix	1
End Polishing Enhancer	1
Distilled Water	1.5
Total Volume	15

11.1.2 Mix and incubate for 20 min at 25°C and 20 min at 72°C in a thermocycler (without heated lid).

Note: *The amount of fragmented DNA can be 0.2-100 ng with an optimal amount of 10-50 ng*

11.2 Adaptor Ligation

11.2.1 Prepare a reaction mix for adaptor ligation according to Table. Add the following reagents to a 0.2 mL PCR tube containing end polished DNA from step 11.1.

Component	Sample (µL)
End polished DNA (from step 11.1)	15
2X Ligation Buffer	17
T4 DNA Ligase	1
Adaptors	1
Total Volume	34

11.2.2 Mix and incubate for 15 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*

(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).

11.3 **Size Selection of Ligated DNA**

If the starting DNA amount is less than 50 ng, size selection is not recommended and alternatively, clean-up of ligated DNA can be performed prior to PCR amplification according to step 11.5 of the protocol.

11.3.1 Resuspend MQ Binding Beads by vortex.

11.3.2 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.

11.3.3 Incubate for 5 minutes at room temperature.

11.3.4 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.

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

11.3.6 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 4 minutes). Carefully remove and discard

the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.3.7 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 min, and then carefully remove and discard the ethanol.

11.3.8 Repeat Step 11.3.7 one time, for total of two washes.

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

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

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

11.3.12 Transfer 11 µL to a new 0.2 mL PCR tube for PCR amplification

11.4 **Clean-Up of Ligated DNA (Optional)**

11.4.1 Resuspend MQ Binding Beads by vortex.

11.4.2 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.

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

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

11.4.5 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 min, and then carefully remove and discard the ethanol.

11.4.6 Repeat Step 11.4.5 two times for total of three washes.

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

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

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

11.4.10 Transfer 11 µL to a new 0.2 mL PCR tube for PCR amplification.

11.5 **Library Amplification**

11.5.1 Prepare the PCR Reactions

Thaw all reaction components including master mix, DNA/RNA free water, primer solution 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	Sample (µL)
HiFi PCR Master Mix (2X)	12.5
Primer U	1
Primer I (or barcode)	1
Adaptor Ligated DNA	10.5
Total Volume	25

Important Note: Use of Primer I included in the kit will generate a singleplexed library. For multiplexed library preparation, replace Primer I with user defined barcodes (Illumina® compatible) instead of Primer I.

11.5.2 Program the PCR Reactions

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

Cycle Step	Temp (˚C)	Time (seconds)	Cycle #
Activation	98	30	1
Cycling	98	20	Variable*
	55	20	
	72	20	
Final Extension	72	120	1

*PCR cycles may vary depending on the input DNA amount. In general, use 10 PCR cycles for 100 ng, 12 cycles for 50 ng, 16 cycles for 5 ng, 18 cycles for 1 ng and 22 cycles for 0.2 ng DNA input. Further optimization of PCR cycle number may be required.

11.6 **Clean-up Amplified Library DNA**

11.6.1 Resuspend MQ Binding Beads by vortex.

11.6.2 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.

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

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

11.6.5 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 min, and then carefully remove and discard the ethanol.

11.6.6 Repeat Step 11.6.5 two times for a total of three washes.

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

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

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

11.6.10 Transfer 20 µL to a new 0.2 mL PCR tube.

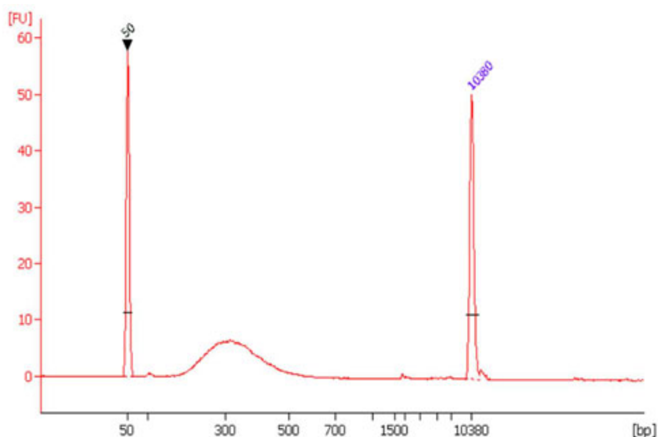
Quality of the prepared library can be assessed using a comparable method. Library fragments should have the correct size distribution (ex: 300 bps at peak size) without adaptors or adaptor- dimers. To check the size distribution, dilute library 5-fold with water and apply it to an Agilent high sensitivity chip. If there is presence of <150 bp adaptor dimers or larger fragments than expected, they should be removed. To remove fragments below 150 bp use 0.8X MQ Binding Beads (ex: add 16 µL of MQ Binding Beads to 20 µL of sample) according to steps 1-10 of section 11.6. Clean-up of Amplified Library DNA. To remove fragments above 500 bps follow steps 1-12 of section 11.3 Size Selection of Ligated DNA.

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

The prepared library DNA can be stored at -20˚C until ready to use for sequencing.

12. ANALYSIS

Typical Results



Size distribution of library fragments. Human placenta DNA was sheared to around 300 bps in peak size and 0.2 ng of DNA was used for DNA library preparation ab185905.

13. 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 >10 ng
	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 Polishing, Adaptor Ligation, size Selection and Amplification
	Improper storage of the kit	Ensure that the kit has not exceeded the expiration date.

Unexpected peak size of bioanalyzer trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected	Improper ratio of MQ Binding	Check if the correct volume of MQ
	Beads to DNA volume in size selection	Binding Beads is added to the DNA solution accordingly. Proper ratios should remove the fragments of unexpected peak size
	Insufficient ligation	Too much and 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 with 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

14. NOTES

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