EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit

Base Catalog # P-2032

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit is designed to enrich a protein (histone or strong binding transcription factor)-specific DNA complex from low input cells/chromatin and to prepare a library for next generation sequencing using Illumina platforms such as Illumina Genome Analyzer II, HiSeq, and MiSeq systems. The innovative working principle, optimized protocol, and components of the kit allow for the capture of targeted protein/DNA complexes with minimized non-specific background levels and for the construction of both non-barcoded (singleplexed) and barcoded (multiplexed) libraries quickly in order to map target protein-DNA interaction regions with less bias and at a high resolution.

Input Amount: For cells**,** in general, the amount can be 2 x 10^3 to 2 x 10^5 cells per reaction. For optimal preparation, the cell input amount should be 1 x 10^5, although cTIP (CUT&Tag In-Place) Sequencing Kit data for modified histones can be obtained with as few as 500 cells. For chromatin isolated from cells or tissues, the amount can be 0.1µg to 5 µg of chromatin per reaction. For optimal preparation, the chromatin input amount should be 2 µg.

Starting Materials: Starting materials can include various mammalian cell samples such as cultured cells from a flask or plate, primary cells, or rare cell populations isolated from blood, body fluid, and fresh/frozen tissues, specific cells sorted from entire cell populations and embryonic cells, etc.

Antibodies: Antibodies should be ChIP-grade in order to recognize the proteins that are bound to DNA or other proteins. If you are using antibodies that have not been validated for ChIP, then an appropriate control antibody such as anti-RNA Polymerase II, anti-H3K4me3, or anti-H3K27me3 should be used to demonstrate that the antibodies are suitable for ChIP.

Internal Controls: Both negative and positive ChIP controls are provided in this kit.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the PCR tubes. 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.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

KIT CONTENTS

For Protein/DNA Capture Reaction

Component	12 reactions Cat. #P-2032-12	24 reactions Cat. #P-2032-24	Storage Upon Receipt
WB (Wash Buffer)	15 ml	30 ml	4°C
LB (Lysis Buffer)	2 ml	4 ml	RT
CB (Capture Buffer)	2 ml	4 ml	RT
NDE (Nuclear Digestion Enhancer)	150 µl	300 µl	RT
PIC (Protease Inhibitor Cocktail)*	15 µl	30 µl	4°C
CEM (Cleavage Enzyme Mix)*	30 µl	60µl	-20°C
Control Antibody (H3K9me3,1 mg/ml)*	5 µl	8 µl	-20°C
Non-Immune IgG (1 mg/ml)*	5 µl	8 µl	4°C
Control Chromatin (100 ng/µl)*	10 µl	20 µl	-20°C
PDB (Protein Digestion Buffer)	500 µl	1000 µl	RT
Proteinase K (10 mg/ml)*	50 µl	100 µl	4°C
Affinity Beads	40 µl	80 µl	4°C

For Library Preparation

Component	12 reactions Cat. #P-2032-12	24 reactions Cat. #P-2032-24	Storage Upon Receipt
10X End Repair Buffer*	30 µl	60 µl	-20°C
End Repair Enzyme Mix*	13 µl	26 µl	-20°C
10X dA Tailing Buffer	30 µl	60 µl	-20°C
dA Tailing Enzyme	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*	2 ml	4 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.*

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

SHIPPING & STORAGE

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit 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™ cTIP (CUT&Tag In-Place)-Sequencing Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The cTIP (CUT&Tag In-Place)-Sequencing Kit and methods of use contain proprietary technologies by EpiGentek.

A BRIEF OVERVIEW

Enrichment of histone or transcription factor (TF)-complexed DNA in vivo followed by next-generation sequencing offers an advantageous tool for studying genome-wide protein-DNA interactions. It allows for the analysis of a specific protein binding to DNA sequences in living cells. Such analysis requires that the method reliably identify true target protein-enriched regions, particularly from limited cell samples. These samples could include rare cell populations isolated from tissues, specific cells sorted from entire cell populations, and primary cultured sub-population cells such as embryonic cells. In addition, the method should ensure that enriched DNA contains minimal background and that the mapping of protein-DNA binding regions is with minimal bias and at high resolution. A major method used for a long time to achieve this goal is chromatin immunoprecipitation followed by sequencing (ChIP-Seq). However, the major limitation for ChIP is that it needs 1) a large amount of input material, cells or tissue, to produce a strong enough signal over background noise; and 2) cross-linking during an initial fixation step. There are several advanced methods available for ChIP-Seq with reduced cell numbers or increased resolution. These methods include ChIP-exo, ChIPmentation. ChIP-exo provides high-resolution mapping, but is time-consuming and requires an ample supply of input cells. While ChIPmentation uses transposase and sequencing-compatible adaptors to enable the integration of ligation during the ChIP process, it follows a traditionally slow (2 day) ChIP procedure and cannot achieve high-resolution mapping.

CUT&RUN (Cleavage Under Target & Release Using Nuclease) and CUT&TAG (Cleavage Under Target & Tagmenation) were developed for mapping protein-DNA interaction with limited biological materials. While they have significantly improved mapping resolution, they are both offered at a high cost. Besides, CUT&RUN needs expensive PA/Mnase fusion protein that has significant A/T sequence bias, causing the target protein-interacted DNA region profiles to be seriously affected by the level of MNase digestion. In addition to the same digestion bias as for CUT&RUN, CUT&TAG is less specific

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

due to off-target accessibility of chromatin caused by the Tn5 transposase enzyme. Therefore, as an improvement, we have developed a new technique: cleavage under target and tag in place for sequencing (CUT&Tag In-Place sequencing) for mapping the genome-wide protein-DNA interactions. Our innovative approach combines the advantages of ChIPmentation, ChIP-exo, and CUT&RUN with the fastest procedures and incorporates it into the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit. This kit has the following features:

- **High enrichment:** Uses a unique nucleic acid cleavage enzyme mix, which has low sequence bias, to simultaneously fragment chromatin and cleave/remove any DNA sequences in both ends of the target protein/DNA complex without affecting DNA occupied by the target protein. Thus, the true target protein-enriched regions can be reliably identified and high-resolution mapping can be achieved.
- **Low input materials:** Robust sonication-free fragmentation, unbound DNA cleavage, and immunocapture are all processed in the same single-tube with on-beads ligation. This method allows for using in both cells and tissues and allows for maximal degradation protection of the target protein with minimal sample loss. As a result, the input cell amount can be as few as 500 cells or chromatin amount can be as low as 50 ng.
- **In place tagging:** The on-beads ligation (in place) of DNA adapters to chromatin prior to DNA purification results in an increase in DNA fragment size, allowing too short fragments (ex: < 70 bps) bound to transcription factors (TF) to be purified for library construction.
- **Minimal background**: Cleavage of unbound DNA sequences in the two (2) end of the target protein/DNA complex in situ enables the minimized immunocapture/sequencing background, allowing data analysis with <10 million reads.
- **Fast, streamlined procedure**: The procedure from cells to library DNA is less than 5 hours.
- **Highly convenient**: The kit contains all required components for each step of the CUT&Tag in-place sequencing, which are sufficient for both protein/DNA capture and captured DNA library preparation, thereby allowing the cTIP (CUT&Tag In-Place)-Sequencing Kit to be the most convenient with reliable and consistent results.

PRINCIPLE & PROCEDURE

The EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit contains all necessary reagents required for carrying out a successful cTIP-Seq starting from mammalian cells. In the reaction, nuclei is isolated from cells. The target protein-DNA complex is bound/captured with the CHIP-grade antibody of interest. With the use of a unique nucleic acid cleavage enzyme mix, chromatin is fragmented, and DNA sequences in both ends of the target protein/DNA complex are cleaved/removed. All the while, the DNA sequence occupied by the target protein is unaffected. The adaptors are ligated to the target protein-bound DNA fragments on the beads. The ligated DNA is then released, purified, and amplified with a high-fidelity PCR mix for library DNA construction. DNA is then cleaned, released, and eluted. Included in the kit are a positive control antibody (anti-H3K9me3), a negative control non-immune IgG, and control chromatin, which can be used to demonstrate the efficacy of the kit and performance at the PCR/bioanalyzer step.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

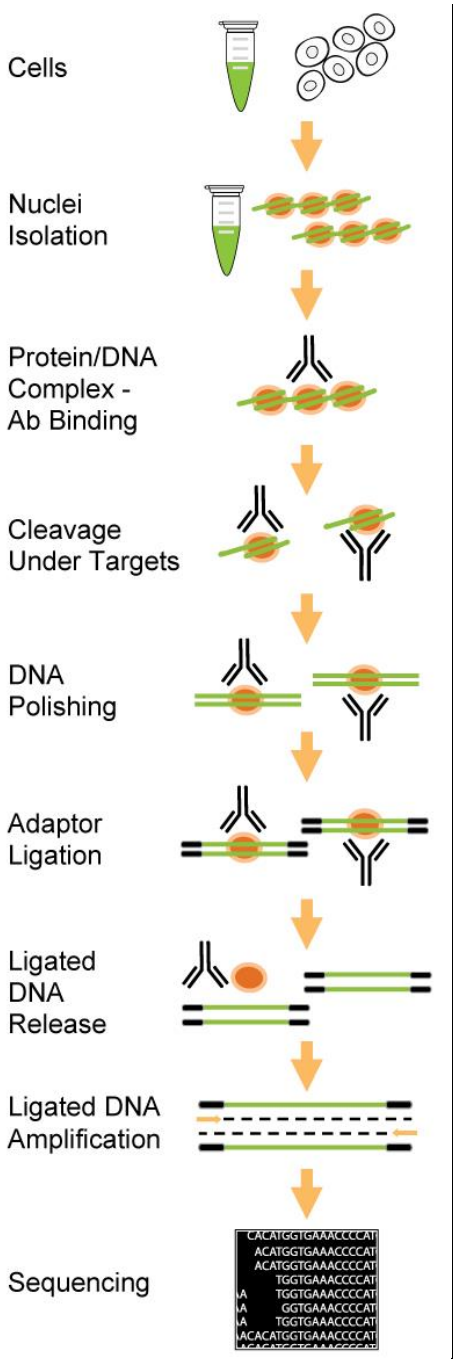

Fig 1. Workflow of the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit.

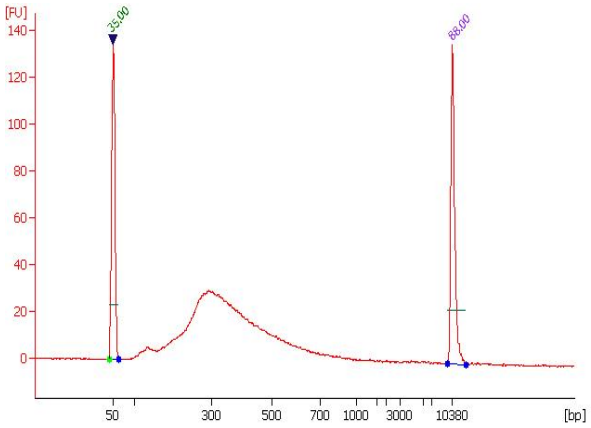

Fig 2. Size distribution of library fragments using the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit: Histone/DNA complex was captured from 500 ng of chromatin isolated from Hela cells by the control antibody (H3K9me3) and used for DNA library preparation. Peak at 295 bps reflects the insert size of mononucleosome (around 150 bps)

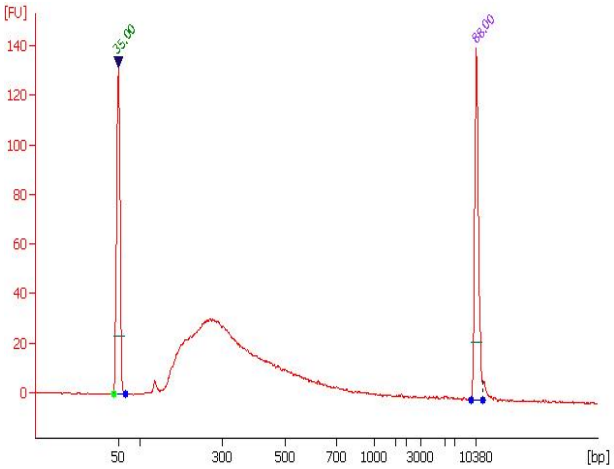

Fig 3. Size distribution of library fragments using the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit: RNA polymerase II/DNA complex was enriched from 50,000 Jurkat cells by anti-RNA polymerase II (PoLII) antibody and used for DNA library preparation. Peak at 280 bps reflects insert size of PoLII occupancy (around 130 bps).

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

Procedure overview and time table

Steps	Required time
Cell lysis and nuclei/chromatin isolation	20 min
Antibody capture and DNA cleavage	100 min
On- beads end polishing and ligation	60 min
DNA release and purification*	40 min
Library amplification and purification	50 min

**Stop point. DNA generated at this step can be stored at -20°C for future amplification use.*

ASSAY PROTOCOL

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

1. Preparation of Working Buffers

a. Prepare **Working Lysis Buffer** by adding 1 µl of **PIC** (**Protease Inhibitor Cocktail)** to every 1 ml of **LB (Lysis Buffer)**
b. Prepare **Working CB (Capture Buffer)** by adding 1 µl of **PIC** (**Protease Inhibitor Cocktail)** to every 1 ml of **CB (Capture Buffer).**

2. Cell Lysis, DNA cleavage, and Immunoprecipitation

a. Isolate or collect the cells according to your own successful methods. These cells could include culture cells from a flask or plate, primary cells, or rare cell populations isolated from blood, body fluid, and fresh/frozen tissues, specific cells sorted from entire cell populations and embryonic cells, etc.

   Note: *If using tissues, the chromatin should be isolated and quantified. EpigenTek provides chromatin preparation kits (See "Related Products" on page 13). The same volume (100 µl) of isolated chromatin can be added for the reaction at Step 2d.*

b. Wash cells with PBS by centrifugation at 1000 rpm for 5 min. Discard the supernatant.
c. Add 110 µl of **Working Lysis Buffer** to re-suspend the cell pellet, and incubate at 4°C for 10 min. Vortex vigorously for 10 sec per 5 min. Meanwhile, prepare antibody-affinity beads solution by adding the reagents to 0.2 ml PCR tubes according to the following and mix well:

Reagents	Sample	Positive Control	Negative Control
CB (**Capture Buffer**)	93-95 µl	96 µl	96 µl
Your Antibodies	1-2 µl	0	0
Control Antibody	0	1 µl	0
Non-Immune IgG	0	0	1 µl
Affinity Beads	4-5 µl	2-3 µl	2-3 µl
Total Volume	**100 µl**	**100 µl**	**100 µl**

Note: *The final amount of each component should be (a) antibodies of interest: 1 µg/tube; (b) positive control antibody: 1 µg/tube; and (c) non-immune IgG: 1 µg/tube.*

*Shake the **Affinity Beads** to completely suspend them before use.*

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

d. Centrifuge the samples at 12,000 rpm (desk-top centrifuge) at 4°C for 10 min and add 110 µl of **CB (Capture Buffer)** into sample. Resuspend the nuclei pellet and transfer 100 µl of the suspended nuclei into PCR tube containing the reagents (if starting cells are less than 1X 10^4, directly transfer 100 µl of cell suspension into the PCR tube without centrifugation after incubation). For the positive and negative controls, add 98 µl of **CB (Capture Buffer)** followed by 5 µl of **Control Chromatin.** Mix and rotate on a rotator or rolling shaker at room temperature for 90 min. Meanwhile, transfer the remaining 10 µ of the suspended nuclei to a 0.2 µl PCR tube and use it as input DNA.

Fragment input DNA according to the following steps:

1. Add 20 µl of **CB (Capture Buffer)** followed by adding 1.5 µl of **NDE (Nuclear Digestion Enhancer),** 1 µl of **CEM** (**Cleavage Enzyme Mix**) and incubate at room temperature for 5 min.
2. Add 3 µl of **Proteinase K** into the tube and incubate at 60°C for 15 min.

Purify input DNA according to the following steps:

1. Resuspend the **MQ Binding Beads** by vortexing. Add 80 µl of resuspended beads to the PCR tube containing the input DNA. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
2. Incubate for 5 min at room temperature to allow the DNA to bind to the beads.
3. Put the PCR tube on an appropriate magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the DNA).*
4. Keep the PCR tube in the magnetic device and add 150 µl of freshly prepared *90% ethanol* to the tube, then carefully remove and discard the ethanol.
5. Repeat Step 4 once for a total of two washes.
6. Resuspend the beads in 13 µl of **Elution Buffer**, and incubate at room temperature for 5 min to release the DNA from the beads.
7. Capture the beads by placing the tube in the magnetic device until the solution is completely clear (about 1 min).
8. Transfer 12.5 µl to a new 0.2 ml PCR tube.

Note: *Quantify the concentration of purified input DNA by a fluorescence method (e.g. Picogreen or Qubit). Dilute the DNA to 1 ng/µl with* ***Elution Buffer*** *and use 12.5 µl of diluted input DNA for the end repair reaction at Step 3b.*

e After the min incubation, Open the tube and add 10 µl of **NDE (Nuclear Digestion Enhancer),** 2 µl of **CEM (Cleavage Enzyme Mix),** and rotate incubate for additional 10 min.

f. Put the tubes on the magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the DNA).*

g Keep the PCR tubes in the magnetic stand and wash each reaction tube twice with 150 µl of **WB (Wash Buffer).** The wash can be performed as follows:

Remove the tubes from the magnetic device (after the solution has been removed) and add **WB (Wash Buffer)** to the reaction tubes. Resuspend the beads by gently pipetting up and down several times. Ensure the pellets are completely resuspended and the beads are not clinging to the pipette tips after pipetting. Place the tubes back in the magnetic stand for 1-2 min to pellet the beads and then remove and discard the solution from each reaction tube.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

3. Target DNA Ligation

a. Prepare **End Repair Reaction Solution** required for each sample according to Table 1:

Table 1A. End Repair Reaction

Component	Volume
10X End Repair Buffer	1.5 µl
End Repair Enzyme Mix	1 µl
Elution Buffer	12.5 µl
Total Volume	**15 µl**

b. After the two washes (Step 2g), add 15 µl of **End Repair Reaction Solution** to each reaction tube. Mix and incubate for 20 min at 25°C in a thermocycler (without heated lid). For the input DNA purified at Step 2d, use 12.5 µl of diluted input DNA solution instead of Elution buffer.

c. Except for the input DNA, put the tubes on the magnetic device until the solution is clear (about 1 min). Carefully remove and discard the solution. Wash each reaction tube once with 150 µl of **WB (Wash Buffer).**

d. For the input DNA, after the end repair reaction, add 30 µl of **MQ Binding Beads,** mix thoroughly by pipetting up and down at least 10 times and follow the same steps 2-8 for input DNA purification at Step 2d.

e. Prepare **dA Tailing Reaction Solution** required for each sample according to Table 2:

Table 2. dA Tailing Reaction

Component	Volume
10X dA Tailing Buffer	1.5 µl
dA Tailing Enzyme	1 µl
Elution Buffer	12.5 µl
Total Volume	**15 µl**

f. Add 15 µl of **dA Tailing Reaction Solution** to each sample. Mix and incubate for 30 min at 37°C in a thermocycler (without heated lid). For input DNA end-repaired and purified at Step 3e use 12.5 µl of the DNA solution instead of **Elution Buffer.**

g. Except for the input DNA, put the tubes on the magnetic device until the solution is clear. Carefully remove and discard the solution. Wash each reaction tube once with 150 µl of **WB (Wash Buffer).**

h. For the input DNA. After the dA tailing reaction, add 30 µl of **MQ Binding Beads,** mix thoroughly by pipetting up and down at least 10 times and follow the same steps 2-8 for input DNA purification at Step 2d.

i. Prepare **Adaptor Ligation Reaction Solution** required for each sample according to Table 3.

Table 3. Adaptor Ligation Reaction

Component	Volume
2X Ligation Buffer	14.5 µl
T4 DNA Ligase	1 µl
Adaptors	1 µl
Total Volume	**16.5 µl**

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

j. For each sample add 12.5 µl of Elution Buffer followed by adding 16.5 µl of **Adaptor Ligation Reaction Solution.** For the input DNA, only add 16.5 µl of **Adaptor Ligation Reaction Solution.** Mix and incubate for 15 min at 25°C in a thermocycler (without heated lid).

k. Put the tubes on the magnetic device until the solution is clear (about 2 min. Carefully remove and discard the solution. *(Caution: Be careful not to disturb or discard the beads that contain the DNA. Meanwhile,* prepare **Protein Digestion Solution** by mixing **Proteinase K** with **PDB** (**Protein Digestion Buffer**) at 1:10 (e.g. 1 µl of **Proteinase K** + 9 µl of **PDB (Protein Digestion Buffer**).

Note: *If there is any liquid on the cap or side wall of the tubes after each incubation step, spin it down to the bottom.*

l. For input DNA, after the ligation reaction, add 60 µl of **MQ Binding Beads,** mix thoroughly by pipetting up and down at least 10 times and follow the same steps 2-8 for input DNA purification at Step 2d.

4. Ligated DNA Release

a. Add 40 µl of **Protein Digestion Solution** to each tube. Mix and incubate at 60°C for 15 min in a thermocycler (without heated lid).

b. Put the tubes on the magnetic device until the solution is clear (about 2 min). Carefully transfer the solution (around 40 µl) from each sample to an unused PCR tube.

c. Resuspend **MQ Binding Beads** by vortexing. Add 60 µl of the resuspended beads to the PCR tubes containing the samples. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

d. Incubate for 5 min at room temperature to allow the DNA to bind to the beads.

e. Put the PCR tubes in the magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the DNA).*

f. Keep the PCR tubes in the magnetic device and add 150 µl of freshly prepared *90% ethanol* to the tubes then carefully remove and discard the ethanol.

g. Repeat Step 4g once for a total of two washes.

h. Resuspend the beads in 13 µl of **Elution Buffer**, and incubate at room temperature for 5 min to release the DNA from the beads.

i. Capture the beads by placing the tubes in the magnetic device until the solution is completely clear (about 1 min).

j. Transfer 12 µl from each sample to a new 0.2 ml PCR tube for PCR amplification.

Note: *Take 1 µl of eluted DNA and quantify the concentration of the ligated DNA by a fluorescence method (e.g. Picogreen or Qubit) so that the library amplification cycles can be determined at the step 5 of library amplification.*

5. Library Amplification

a. Prepare the PCR reactions.

Thaw all reaction components, including master mix, primer solution, and DNA template. Mix well by vortexing briefly. Keep the components on ice while in use and return to -20˚C immediately following use. Add the components into the sample and input PCR tubes according to the following table:

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

Component	Size (µl)
2X HiFi PCR Master Mix	12.5 µl
Primer U	1 µl
Primer I	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** (#P-1060, EpiGentek). You can also add user-defined barcodes (Illumina compatible) instead of **Primer I**.*

b. Program the PCR reactions.

Place the reaction tubes in the instrument and set the PCR conditions as follows:

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	20 sec 20 sec 20 sec	Variable*
Final Extension	72°C	2 min	1

** PCR cycles may vary depending on the ligated DNA concentration. In general, use 14 cycles for 8 ng, 17 cycles for 2 ng, and 21 cycles for 0.5 ng of the ligated DNA. Further optimization of PCR cycle number may be required.*

6. Clean-Up of Amplified Library DNA

a. Resuspend **MQ Binding Beads** by vortexing.
b. Add exactly 25 µl (1X) of the resuspended beads to the PCR tubes of the amplification reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 min at room temperature to allow the DNA to bind to the beads.
d. Put the PCR tubes in the magnetic stand until the solution is clear (about 2 min). Carefully remove and discard the supernatant. (Caution: Be careful not to disturb or discard the beads that contain the DNA.)
e. Keep the PCR tubes in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tubes, then carefully remove and discard the ethanol.
f. Repeat Step 6e once for a total of two washes.
g. Resuspend the beads in 12 µl of **Elution Buffer**, and incubate at room temperature for 5 min to release the DNA from the beads.
h. Capture the beads by placing the tubes in the magnetic stand until the solution is completely clear (about 1 min).
i. Transfer 12 µl from each sample to a new 0.2 ml PCR tube.

Quality of the prepared libraries, including the positive and negative controls, can be assessed using an Agilent® Bioanalyzer® or other comparable methods. Library fragments should have the correct size distribution (e.g., 200-400 bps at peak size) without adaptors or adaptor-dimers.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

To check the size distribution, dilute the 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** according to sub-steps a - i of Step 6 of "Clean-Up of Amplified Library DNA".

Store the prepared library at -20ºC until ready to use for sequencing.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Little or no library DNA generated from sample.	Insufficient amount of qualified cells.	The cells used for the reaction may be broken, causing loss of chromatin. Check if the cells are intact by microscope.
	Poor enrichment with antibody; some antibodies used in ChIP might not efficiently recognize fixed protein.	Increase the antibody amount and use ChIP-grade antibodies validated for use in ChIP.
	Inappropriate DNA fragmenting condition.	**Cleavage Enzyme Mix** (**CEM**) may be degraded due to improper temperature storage. Ensure the proper storage condition for this component. Chromatin is from specific cell/tissue types. Under this condition, the cleavage should be optimized to allow DNA fragment size to be < 200 bps.
	Incorrect temperature and/or insufficient time during DNA release.	Ensure the incubation times and temperatures described in the protocol are followed correctly.
	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 DNA End Polishing, Adaptor Ligation, and Amplification.
	Improper storage of the kit.	Ensure that the kit has not exceeded the expiration date. Standard shelf-life, when stored properly, is 6 months from date of receipt.
No difference in library DNA intensity between negative and positive control tubes	Insufficient washing.	Check if washing recommendations at each step are performed according to the protocol. If the signal intensity in the negative control is still high, washing stringency can be increased in the following ways: 1. Increase wash time at each wash step: after adding **WB** (**Wash Buffer**), leave it in the tubes for 3-4 min and then remove it. 2. Add an additional one wash with **WB** (**Wash Buffer**), The provided volume of this component is sufficient for 4 extra washes for each sample.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

	Too many PCR cycles: Plateau phase of amplification caused by excessive number of cycles in endpoint PCR may mask the difference of signal intensity between negative and positive control.	Decrease the number of PCR cycles to keep amplification at the exponential phase. This will reduce high background in endpoint PCR and allow differences in amplification to be seen. Real time PCR is another choice in such cases.
Unexpected peak size of Agilent® Bioanalyzer® trace: presence of <150 bp adaptor dimers or presence of larger fragments than expected	Improper ratio of **MQ Binding Beads** to DNA volume in size selection.	Check if the correct volume of **MQ Binding Beads** is added to DNA solution accordingly. Proper ratios should remove the fragments with unexpected peak sizes.
	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

Chromatin Preparation

P-2001 ChromaFlash™ Chromatin Extraction Kit
P-2023 ChromaFlash™ Chromatin Isolation/Shearing Kit

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 Tissue (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™ ChIP Ultra Kit
P-2030 EpiNext™ ChIP-Seq High Sensitivity Kit

PCR Analysis

P-1029 EpiQuik™ Quantitative PCR Kit

DNA Library Prep

P-1051 EpiNext™ DNA Library Preparation Kit (Illumina)
P-1053 EpiNext™ High Sensitive DNA Library Prep Kit (Illumina)

NGS Barcode

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

For ChIP-grade antibodies, search "chip-grade" at www.epigentek.com.

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com