Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina

HD102

Vazyme

Instruction for Use

Version 25.2

Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina is a protein-DNA interaction assay kit specially designed for the Illumina platform. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a new method for protein-DNA interaction research. Guided by the antibody, the fused pG-MNase is tethered to the target protein, enabling precise DNA cleavage near the binding site. With an optimized reaction system and library preparation workflow, this kit offers multiple advantages over conventional chromatin immunoprecipitation followed by sequencing (ChIP-seq), including a high success rate, excellent antibody compatibility, short operation time, and easy procedures, making it especially suitable for such fields as early embryonic development, stem cells, cancer, and epigenetics. All the components provided in the kit have undergone rigorous quality control and functional testing to ensure optimal stability and repeatability.

02/Components

	Components	HD102-01 (24 rxns)
BOX 1	ConA Beads Pro	260 µl
BOX 2	FastPure gDNA Mini Columns	24
	Collection Tubes 2 ml	24
	Buffer GDP	24 ml
	Buffer GW	4 ml
BOX 3	pG-MNase Enzyme	30 µl
	MNase Dilution Buffer	2 × 1.3 ml
	5% Digitonin	1.3 ml
	10 × Binding Buffer	800 µl
	10 × Wash Buffer	12 ml
	Antibody Buffer(-)	2 × 1.2 ml
	$CaCl_2$	48 µl
	2 × Stop Buffer(-)	2 × 1.2 ml
	Spike in DNA (5 ng/µl)	24 µl
	DNA Damage Repair Enzyme	48 µl
	End Prep Buffer	240 µl
	End Prep Enzyme	120 µl
	Rapid Ligation Buffer	600 µl
	Rapid DNA Ligase	120 µl
	VAHTS HiFi Amplification Mix	600 µl
	PCR Primer Mix3 for Illumina	120 µl

▲ The colors in the table correspond to the cap color of each component.
FastPure gDNA Mini Columns: DNA adsorption;
Collection Tubes 2 ml: Filtrate collection;
Buffer GDP: DNA binding buffer;
Buffer GW: Washing buffer. Add absolute ethanol as indicated on the bottle before use.

03/Storage

BOX 1: Store ConA Beads Pro at 2 ~ 8°C and ship on ice pack;

BOX 2: Store at 15 ~ 25°C and ship at room temperature;

BOX 3: Store 5% Digitonin at -30 ~ -15°C; it can be stored at room temperature (15 ~ 25°C) for one week;
Store the other components at -30 ~ -15°C;
Ship at ≤0°C.

04/Applications

Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina is designed for protein-DNA interaction research using 5,000 - 500,000 mammalian cells. Yeast and plant tissues should be pretreated before use.

05/Self-prepared Materials

Reagents

Antibodies: Primary antibody and secondary antibody (optional).

Protease inhibitor: Roche Complete Protease Inhibitor EDTA-Free Tablets (Sigma-Aldrich #5056489001).

Magnetic beads for purification: VAHTS DNA Clean Beads (Vazyme #N411).

DNA Adapter:

VAHTS DNA Adapters Set 3 - Set 6 for Illumina (Vazyme #N805/N806/N807/N808); 24 different single indexes are provided in each set, with 96 different indexes provided in 4 sets in total.

VAHTS Multiplex Oligos Set 4 - Set 5 for Illumina (Vazyme #N321/N322); 96 different dual indexes are provided in each set, with 384 different indexes provided in 2 sets in total.

Use an appropriate adapter kit according to the sample quantity.

Others: Absolute ethanol and ddH_2O.

Instruments and Consumables

PCR instrument, rotary mixer, magnetic rack, Agilent Technologies 2100 Bioanalyzer or other equivalent products; low-adsorption EP tube, PCR tube, etc.

06/Notes

The experimental process can be adjusted based on various factors, including the sample, target protein, etc. Please read the following precautions carefully.

Contact Vazyme Technical Support for assistance in case of any issues during operation: support@vazyme.com.

06-1/About Magnetic Beads

◇ Two kinds of magnetic beads are used in the experiment. ConA Beads Pro is for cell binding, and VAHTS DNA Clean Beads (Vazyme #N411) is recommended for library purification. Correctly distinguish the magnetic beads before use.

◇ General precautions for magnetic beads:

▲ Store the beads at 2 ~ 8°C. Do not freeze at -30 ~ -15°C.

▲ Equilibrate the beads to room temperature and mix well before use.

◇ About ConA Beads Pro:

▲ Do not mix by vigorously vortexing or pipetting after incubating ConA Beads Pro with cells to prevent cell loss.

▲ Avoid prolonged exposure of beads or bead-cell complexes to air to prevent adverse results.

▲ Avoid high-speed centrifugation or keeping the tube on the magnetic rack for a long time to prevent bead aggregation.

▲ Bead aggregation or adsorption is normal and related to the cell types and inputs. No adverse effects will be caused as long as the beads or the complexes are in the reaction solution. Please gently flick the tube instead of vortexing or pipetting in case of bead aggregation or adsorption.

◇ About DNA Clean Beads:

▲ Transfer the supernatant when the solution is clear without disturbing the beads.

▲ Wash beads with freshly prepared 80% ethanol on the magnetic rack.

▲ Air-dry the beads on the magnetic rack until their surface becomes matte brown. Over-drying may affect the results.

06-2/About Sample Preparation and Antibody Selection

◇ Discard the medium and harvest the suspension cells after centrifugation. Most adherent cells can be digested by trypsin. Discard the supernatant and harvest the cells after centrifugation. Since trypsin digestion may affect the binding efficiency of ConA Beads Pro to certain cell lines, please adjust the harvesting method according to the specific cell type.

◇ Use trypan blue staining to assess cell viability and ensure that the cell viability is >90%. Random fragmentation often occurs in dead cells or cells with low viability due to chromatin relaxation and the subsequent exposure of naked DNA, which leads to high background noise.

◇ Light cross-linking is recommended for some special low-abundance target proteins to obtain optimal results.

◇ It is recommended to set up a positive control group and a negative control group in the experiment. High-abundance histone proteins, such as H3K4me3, are recommended as the positive control, while a non-specific antibody (e.g., IgG) is recommended as the negative control.

◇ Chromatin immunoprecipitation (ChIP)-grade primary antibodies are recommended. If unavailable, immunofluorescence (IF)-grade antibodies are recommended.

◇ Secondary antibody incubation is optional for some special target proteins. An unmodified secondary antibody with high affinity to Protein G is recommended.

06-3/About Column-Based Extraction Reagents

◇ Add absolute ethanol to Buffer GW to the indicated volume on the bottle before first use. Store at room temperature.

◇ Buffer GDP precipitates when stored at low temperature. Equilibrate it to room temperature or incubate it in a 37°C water bath until the precipitation is completely dissolved. Mix well before use.

◇ Perform extraction at room temperature (15 ~ 25°C).

06-4/About Spike in DNA

◇ The Spike in DNA provided in the kit is a 300 bp fragment from *E. coli* λDNA, which is used for inter-group normalization.

◇ Dilute the Spike in DNA with ddH_2O and use it immediately after preparation. Quantify the diluted Spike in DNA before use to ensure accurate addition.

◇ The Spike in DNA sequence is as follows. The sequence information is also available on our website (www.vazyme.com).

ATAACTCAATGTTGGCCTGTATAGCTTCAGTGATTGCGATTCGCCTGTCTCTGCCTAAT
CCAAACTCTTTACCCGTCCTTGGGTCCCTGTAGCAGTAATATCCATTGTTTCTTATATAA
AGGTTAGGGGGTAAATCCCGGCGCTCATGACTTCGCCTTCTTCCCATTTCTGATCCTC
TTCAAAAGGCCACCTGTTACTGGTCGATTTAAGTCAACCTTTACCGCTGATTCGTGGAA
CAGATACTCTCTTCCATCCTTAACCGGAGGTGGGAATATCCTGCATTCCCGAACCCATC
GACGA

06-5/About Adapter

◇ Library quality is related to the adapter quality and input. Excessive adapters lead to adapter residues and adapter dimerization, while inadequate adapters lead to a low library yield.

◇ Dilute the adapter with ddH_2O and use it immediately after preparation.

◇ Dilute the adapter at a ratio between 1:10 - 1:50 for 10,000 - 500,000 cells and between 1:50 - 1:100 for less than 10,000 cells. Please adjust the dilution ratio based on different target proteins and cell inputs.

06-6/About Cell Inputs and Amplification Cycles

◇ The kit is compatible with 5,000 - 500,000 cells, and 10,000 - 100,000 cells are recommended for the first experiment. The library concentration is related to the cell types and inputs, target protein, and antibody. Please adjust the amplification cycles according to the actual conditions.

The recommended amplification cycles for the high-abundance target protein (such as H3K4me3) are as follows:

Cell Inputs	Cycles	Library Yield (Qubit Assay)
5,000	17 - 20	5 - 30 ng/μl
10,000	13 - 16	
100,000	12 - 14	10 - 60 ng/μl
500,000	10 - 12	

◇ Low cell input is not recommended; if necessary, please optimize the procedure accordingly.

◇ For low-abundance target proteins, it is recommended to increase the number of PCR cycles by 2 - 4 based on the table above. Please use an appropriate number of PCR cycles to avoid over-amplification, amplification bias, PCR duplicates, chimeric products, and amplification-induced mutations.

06-7/About Library Quality Control

◇ Library concentration analysis

There are two methods for library quantification: one is based on dsDNA fluorescent dyes, such as PicoGreen and Equalbit dsDNA HS Assay Kit (Vazyme #EQ121); the other is qPCR-based quantification, such as VAHTS Library Quantification Kit for Illumina 2.0 (Vazyme #NQ107).

◇ Library size distribution analysis

Library size distribution analysis can be performed using Agilent 2100 Bioanalyzer or other equivalent products.

06-8/Additional Precautions

◇ Thaw all the components at the appropriate temperature and mix well before use.

◇ Store all the components under appropriate conditions.

◇ It is recommended to use pipette tips with filters and change the pipette tips when aspirating different samples.

◇ It is recommended to physically isolate the PCR reaction preparation area from the PCR product purification area to avoid aerosol contamination.

07/Experiment Process

07-1/Buffer Preparation

▲ The following volumes are calculated based on an individual sample. Please scale the preparation according to the actual number of samples.

1. Binding Buffer: Dilute 30 µl of 10 × Binding Buffer with 270 µl of ddH_2O and mix well.
2. Wash Buffer: Add 100 µl of 50 × Protease Inhibitor to 500 µl of 10 × Wash Buffer. Add 4.4 ml of ddH_2O and mix well.
   ▲ 50 × Protease Inhibitor: Add one protease inhibitor tablet (Sigma-Aldrich, #5056489001) in 1 ml of ddH_2O and mix well. Store at -30 ~ -15°C.
   ▲ The prepared Wash Buffer can be stored at 4°C overnight.
3. Antibody Buffer: Add 1 µl of 5% Digitonin to 100 µl of Antibody Buffer(-), mix well, and place on ice for pre-cooling.
4. Dig-wash Buffer: Add 38 µl of 5% Digitonin to 3.8 ml of the Wash Buffer prepared in Step 2 and mix well.
   ▲ Digitonin is toxic. Ensure proper personal protection when preparing the solution. The buffer cannot be stored for a long time following the addition of digitonin. Use it immediately after preparation.

5. Stop Buffer: Add 1 µl of 5% Digitonin to 100 µl of 2 × Stop Buffer(-). Add an appropriate amount of Spike in DNA according to the cell inputs, and place it on ice for later use.
   ▲ Spike in DNA can be diluted with ddH_2O. Use it immediately after preparation.
   ▲ Spike in DNA is used for inter-group normalization. Using H3K4me3 detection in K562 cells as an example, the recommended input amount of spike-in DNA is shown in the table below:

Cell Inputs	Spike in DNA Inputs
5,000 - 10,000	1 pg
100,000	10 pg
500,000	50 pg

6. Add absolute ethanol to Buffer GW to the indicated volume on the bottle before first use.

HD102-01	Volume of Absolute Ethanol (ml)
Buffer GW	20

07-2/ConA Beads Pro Treatment

1. Add 100 µl of Binding Buffer to a 1.5 ml tube.
2. Mix well ConA Beads Pro by pipetting. Add 10 µl of ConA Beads Pro to the Binding Buffer from Step 1 and mix well. Place the 1.5 ml tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min).
3. Remove the tube from the magnetic rack, add 100 µl of Binding Buffer, and mix well by pipetting.
4. Place the tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min). Add 10 µl of Binding Buffer to resuspend ConA Beads Pro.

07-3/Cell Harvesting

▲ Perform all the steps before cell permeabilization at room temperature to minimize cell stress. Avoid vigorous vortexing during the experiment.

1. Harvest and count cells at room temperature.
2. Transfer the required number of cells to a 1.5 ml tube. Centrifuge at 2,500 rpm (600 × g) for 5 min at room temperature, and discard the supernatant.
3. Resuspend the cells in 500 µl of Wash Buffer at room temperature. Centrifuge at 2,500 rpm (600 × g) for 5 min, and discard the supernatant.
4. Resuspend the cells with 100 µl of Wash Buffer for each sample.

07-4/Cell and ConA Beads Pro Incubation

1. Transfer 100 µl of cells to the tube containing the prepared ConA Beads Pro. Mix well by inversion and incubate for 10 min at room temperature, inverting the tube 2 - 3 times during incubation.
   ▲ Do not mix by pipetting or vortexing after the incubation of cells with ConA beads Pro.
2. Briefly centrifuge (<100 × g) to collect the reaction mix. Place the tube on the magnetic rack. Discard the supernatant when the solution is clear (about 2 min).
   ▲ Avoid magnetic bead aggregation at the tube bottom due to prolonged centrifugation.

07-5/Primary Antibody Incubation

1. Add 100 µl of pre-cooled Antibody Buffer to each tube to resuspend the cell-bead complex.
2. Add the antibody to the EP tube at a concentration specified in the antibody manual, and mix well by inversion.
3. Briefly centrifuge to collect the reaction solution (avoid magnetic bead aggregation at the tube bottom due to prolonged centrifugation), and incubate at 4°C overnight.
   ▲ It is recommended to include both positive and negative controls in the experiment.

07-6/pG-MNase Enzyme Incubation

1. Add 1 µl of pG-MNase Enzyme to 100 µl of MNase Dilution Buffer. Then add 1 µl of the diluted enzyme to 100 µl of Dig-wash Buffer to prepare the pG-MNase Enzyme mixture. Mix by inversion and place on ice for later use.
   ▲ Dilute the pG-MNase enzyme immediately before use to ensure optimal cleavage activity. To maintain its stability, it is recommended to aliquot the pG-MNase enzyme upon first use and store the aliquots at -30 ~ -15°C.
2. Briefly centrifuge the tube from 07-5/Primary Antibody Incubation to collect the reaction solution. Place the tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min).
3. Add 800 µl of Dig-wash Buffer to the tube and mix the reaction solution well by inversion.
4. Briefly centrifuge to collect the reaction solution. Place the tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min).
5. Repeat Steps 3 - 4 (twice in total).
6. Add 100 µl of the pG-MNase Enzyme mixture from Step 1 and mix well by inversion.
7. Incubate with rotation at 4°C for 1 h.

07-7/Fragmentation

1. Add 2 µl of $CaCl_2$ to 98 µl of Dig-wash Buffer. Mix by inversion and place on ice for later use.
2. Briefly centrifuge the tube from 07-6/pG-MNase Enzyme Incubation to collect the reaction mix. Place the tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min).
3. Add 800 µl of Dig-wash Buffer to the tube and mix well by inversion.
4. Briefly centrifuge to collect the reaction mix. Place the tube on the magnetic rack and discard the supernatant when the solution is clear (about 2 min).
5. Repeat Steps 3 - 4 (twice in total).
6. Add 100 µl of the prepared $CaCl_2$ mix from Step 1 and mix well by inversion.
7. Immediately place the tube on ice and incubate for 60 - 90 min. Mix by inversion 2 - 3 times during incubation.

   ▲ Fragmentation conditions vary across different target proteins and should be adjusted accordingly. Prolonged incubation is recommended for low-abundance target proteins, such as 60 min for common histones (e.g., H3K4me3) and 90 min for transcription factors (e.g., CTCF).

07-8/Fragmentation Termination and DNA Fragment Release

1. Add 100 µl of Stop Buffer to the tube from 07-7/Fragmentation, and mix well by inversion.
2. Incubate the tube in a 37°C water bath for 10 - 30 min.

   ▲ Do not disturb the tube during incubation to allow the release of DNA fragments.
3. Centrifuge at 12,000 rpm (13,400 × g) for 5 min.
4. Place the EP tube on the magnetic rack. Transfer the supernatant to a new 1.5 ml tube when the solution is clear (30 sec - 2 min).

   ▲ The supernatant from Step 4 contains chromatin-enriched products and can be stored at -30 ~ -15°C for 7 days.

07-9/DNA Extraction

1. Add 1 ml of Buffer GDP to the 1.5 ml tube from 07-8/Fragmentation Termination and DNA Fragment Release, mix well by vortexing, and incubate at room temperature for 10 min, mixing 2 - 3 times by inversion.
2. Briefly centrifuge to collect the reaction solution. Place the FastPure gDNA Mini Columns in a Collection Tube 2 ml. Transfer 650 µl of the reaction solution to the spin column, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
3. Discard the filtrate and place the spin column in the Collection Tube. Transfer the rest of the reaction solution to the spin column, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.

4. Discard the filtrate and place the spin column in the Collection Tube. Add 700 µl of Buffer GW (supplemented with absolute ethanol) to the spin column. Centrifuge at 12,000 rpm (13,400 × g) for 60 sec.

   ▲ Add Buffer GW along the wall of the spin column, or invert the column with the cap securely closed 2 - 3 times and collect the Buffer GW.

5. Discard the filtrate and place the spin column in the Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min.

6. Air-dry the spin column at room temperature for 2 - 5 min to ensure DNA purity.

7. Place the spin column in a new 1.5 ml EP tube. Add 22 µl of ddH_2O to the center of the column, and incubate for 2 min.

8. Centrifuge at 12,000 rpm (13,400 × g) for 2 min. Discard the spin column and store the extracted product at -30 ~ -15°C.

   ▲ The product from Step 8 contains the target DNA fragments and can be stored at -30 ~ -15°C for 7 days.

07-10/Library Preparation

Step 1: DNA Damage Repair & End Preparation

1. Thaw End Prep Buffer on ice and mix well by inversion before use. Prepare the following reaction mix in a PCR tube:

Components	Volume	
DNA Fragments	15 - 20 µl	
DNA Damage Repair Enzyme	2 µl	☐
End Prep Enzyme	5 µl	■
End Prep Buffer	10 µl	■
ddH_2O	To 65 µl	

▲ Mix well by pipetting, and briefly centrifuge to collect the reaction mix to the tube bottom.

2. Place the PCR tube in the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
30°C	20 min
65°C	15 min
4°C	Hold

Step 2: Adapter Ligation

1. Dilute the adapter to an appropriate concentration based on the cell inputs. Please refer to 06-5/About Adapter.

2. Thaw Rapid Ligation Buffer on ice and mix well by inversion before use.

Prepare the following reaction mix in a PCR tube:

◇ Adapter ligation reaction mix for full-length adapter (Vazyme #N805/N806/N807/N808, single index)

Components	Volume	
End Preparation Product	65 μl	
Rapid Ligation Buffer	25 μl	■
Rapid DNA Ligase	5 μl	■
DNA Adapter X	5 μl	
In Total	100 μl	

◇ Adapter ligation reaction mix for stubby adapter (Vazyme #N321/N322, dual index)

Components	Volume	
End Preparation Product	65 μl	
Rapid Ligation Buffer	25 μl	■
Rapid DNA Ligase	5 μl	■
DNA Adapter-S for Illumina	5 μl	
In Total	100 μl	

▲ Mix well by pipetting, and briefly centrifuge to collect the reaction mix to the tube bottom.

3. Place the PCR tube in the PCR instrument and perform the following program:

Temperature	Time
Heated lid 105°C	On
20°C	15 min
4°C	Hold

4. Purify the ligation product using VAHTS DNA Clean Beads (Vazyme #N411):

a. Equilibrate the VAHTS DNA Clean Beads to room temperature and mix well by vortexing.

b. Add 60 μl of VAHTS DNA Clean Beads to 100 μl of adapter ligation product and mix well by pipetting or vortexing. Incubate at room temperature for 5 min.

c. Briefly centrifuge and place the tube on the magnetic rack. Discard the supernatant when the solution is clear (about 5 min). Do not disturb the beads!

d. Keep the PCR tube on the magnetic rack and wash the beads with 200 μl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, then carefully discard the supernatant.

e. Repeat Step d (twice in total).

f. Keep the PCR tube on the magnetic rack and air-dry the beads for 5 min.

g. Remove the tube from the magnetic rack and elute with 22 μl of ddH_2O. Mix well by pipetting or vortexing and incubate at room temperature for 5 min.

h. Briefly centrifuge the tube and place the tube on the magnetic rack. Transfer 20 μl of supernatant to a new EP tube when the solution is clear (about 5 min). Store at -30 ~ -15°C.

▲ The adapter ligation product from Step h can be stored at -30 ~ -15°C for 7 days. Avoid repeated freeze-thaw cycles.

Step 3: Library Amplification

1. Thaw PCR Primer Mix 3 for Illumina and VAHTS HiFi Amplification Mix, mix well by inversion, and prepare the following reaction in a PCR tube:

◇ Library amplification reaction mix for full-length adapter (Vazyme #N805/N806/N807/N808, single index)

Components	Volume
Purified Adapter Ligation Product	20 μl
PCR Primer Mix3 for Illumina	5 μl
VAHTS HiFi Amplification Mix	25 μl
In Total	50 μl

▲ PCR Primer Mix3 for Illumina is from Vazyme #HD102.

◇ Library amplification reaction mix for stubby adapter (Vazyme #N321/N322, dual index)

Components	Volume
Purified Adapter Ligation Product	20 μl
VAHTS i5 PCR Primers	2.5 μl
VAHTS i7 PCR Primers	2.5 μl
VAHTS HiFi Amplification Mix	25 μl
In Total	50 μl

▲ VAHTS i5/i7 PCR Primers are from Vazyme #N321/N322.

▲ Mix well by pipetting, and centrifuge briefly to collect the reaction mix to the bottom of the tube.

2. Place the PCR tube in the PCR instrument and perform the following program:

Temperature	Time	Cycles
95°C	3 min	
98°C 60°C	10 sec 5 sec	10 - 20
72°C	1 min	
4°C	Hold	

▲ Select an appropriate number of amplification cycles based on the target protein.

3. Purification using VAHTS DNA Clean Beads (Vazyme #N411):

a. Equilibrate the VAHTS DNA Clean Beads to room temperature and mix well by vortexing.

b. Add 45 μl of VAHTS DNA Clean Beads into 50 μl of library amplification product and mix well by pipetting or vortexing. Incubate at room temperature for 5 min.

c. Briefly centrifuge and place the tube on the magnetic rack. Discard the supernatant when the solution is clear (about 5 min). Do not disturb the beads!

d. Keep the PCR tube on the magnetic rack and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the beads. Incubate at room temperature for 30 sec, then carefully discard the supernatant.

e. Repeat Step d (twice in total).

f. Keep the PCR tube on the magnetic rack and air-dry the beads for 5 min.

g. Remove the tube from the magnetic rack and elute with 22 µl of ddH_2O. Mix well by pipetting or vortexing and incubate at room temperature for 5 min.

h. Briefly centrifuge the tube and place it on the magnetic rack. Transfer 20 µl of supernatant to a new EP tube when the solution is clear (about 5 min).

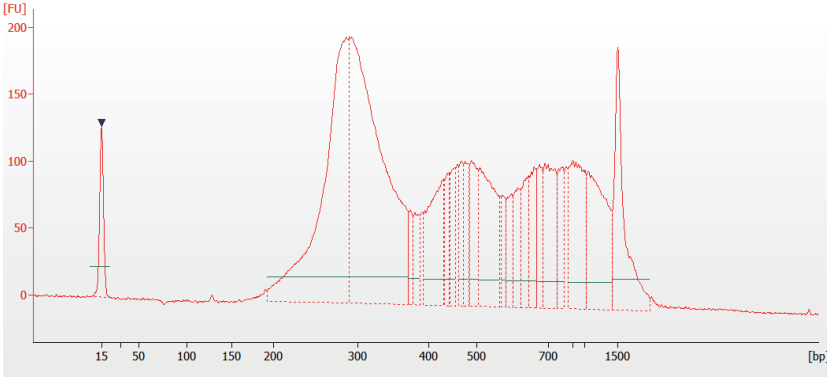
▲ The product from Step h can be stored at -30 ~ -15°C. Avoid repeated freeze-thaw cycles during long-term storage.

07-11/Library Quality Control

1. Library size distribution analysis:

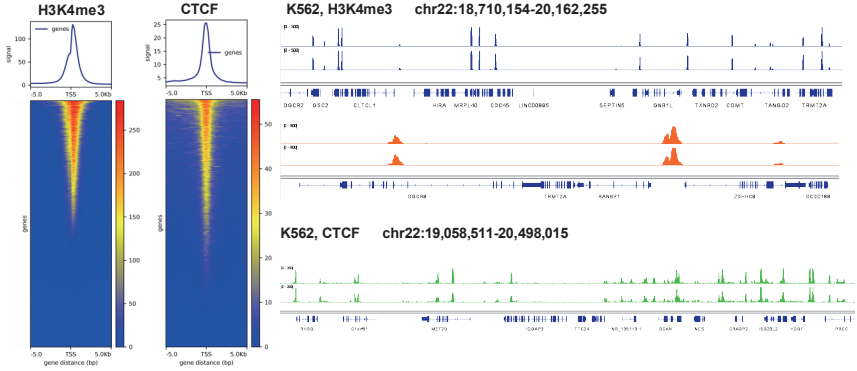
Please refer to 06-7/About Library Quality Control.

Use 100,000 K562 cells for CUT&RUN library preparation (H3K4me3). The library size distribution is as follows:



2. Transcription start site (TSS) enrichment and integrative genomics viewer (IGV):

Use 100,000 K562 cells for CUT&RUN library preparation (H3K4me3 and CTCF). The TSS enrichment and IGV view are as follows:



08/FAQ & Troubleshooting

◇What species can CUT&RUN be applied to?

Hyperactive pG-MNase CUT&RUN Assay Kit for Illumina is designed for protein-DNA interaction research using 5,000 - 500,000 mammalian cells. Yeast and plant tissues should be pretreated before use.

◇What are ConA magnetic beads mainly used for?

ConA beads are used to immobilize cells by binding to glycoproteins on the cell surface, enabling adsorption and visualization of cells, and reducing cell loss.

◇Are CUT&RUN products only suitable for sequencing on Illumina platforms?

The PCR Primer Mix3 for Illumina provided in the kit is suitable for the Illumina platform. If a different sequencing platform is required, please replace the adapter and amplification primer with appropriate equivalents.

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