Hyperactive pG-MNase CUT&RUN Assay Kit for PCR/qPCR

HD101

Vazyme

Instruction for Use

Version 23.2

Vazyme

Contents

01/Product Description

Hyperactive pG-MNase CUT&RUN Assay Kit for PCR/qPCR is developed specially for studying protein-DNA interactions. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a new method for studying protein-DNA interactions. By fusing Protein G with transposase, under the guidance of the antibody, it allows accurate targeting of the target protein and cutting of the DNA sequence near the target site. With an optimized reaction system and workflow, this kit offers multiple advantages over conventional ChIP-qPCR, including high success rate, excellent antibody compatibility, short turnaround time, and ease of use, making It is especially suitable for such fields as early embryonic development, stem cells, cancer, and epigenetics. All reagents in this kit have undergone strict quality control and function testing to ensure the optimal stability and repeatability of experimental results.

02/Components

		Components	HD101-01 (24 rxns)	HD101-02 (48 rxns)
BOX 1	■	ConA Beads Pro	260 µl	520 µl
BOX 2		FastPure gDNA Mini Columns	24	48
		Collection Tubes 2 ml	24	48
		Buffer GDP	24 ml	48 ml
		Buffer GW	4 ml	8 ml
BOX 3	■	pG-MNase Enzyme	30 µl	60 µl
	□	MNase Dilution Buffer	2 × 1.3 ml	4 × 1.3 ml
	■	5% Digitonin	1.3 ml	2 × 1.3 ml
	■	10 × Binding Buffer	800 µl	2 × 800 µl
		10 × Wash Buffer	12 ml	24 ml
	■	Antibody Buffer(-)	2 × 1.2 ml	4 × 1.2 ml
	■	$CaCl_2$	48 µl	96 µl
	■	2 × Stop Buffer(-)	2 × 1.2 ml	4 × 1.2 ml
	■	Spike in DNA (5 ng/µl)	24 µl	48 µl
	□	2 × ChamQ Universal SYBR qPCR Master Mix	2 × 800 µl	4 × 800 µl
	■	Spike in Primer F (10 µM)	40 µl	80 µl
	■	Spike in Primer R (10 µM)	40 µl	80 µl
	■	Positive Control Primer F (10 µM)	15 µl	30 µl
	■	Positive Control Primer R (10 µM)	15 µl	30 µl
	□	RNase-free ddH_2O	500 µl	1 ml

▲ The color marked in the components table represents the color of tube cap of each component;
FastPure gDNA Mini Columns: DNA absorption columns;
Collection Tubes 2 ml: Collect tubes for filtrate;
Buffer GDP: DNA binding buffer;
Buffer GW: Washing buffer. Add absolute ethanol by the indicate volume on the bottle before use.

03/Storage

BOX 1: Store ConA Beads Pro at 2 ~ 8°C and adjust the transportation method according to the destination.

BOX 2: Store at 15 ~ 25°C and transport 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 2 × ChamQ Universal SYBR qPCR Master Mix at -30 ~ -15°C and protect from light.

Store the other components at -30 ~ -15°C.

Transport at ≤0°C.

04/Applications

This product is intended for studying protein-DNA interactions in mammalian cells with 5,000 - 500,000 input cells. Yeast, plant tissues and cells should be treated before proceeding with the assay using this kit.

05/Self-prepared Materials

Reagents

Antibodies: Primary antibody and secondary antibody (optional).

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

Others: Ethanol absolute and ddH_2O.

Instruments and Consumables

Rotary mixer;

Magnetic stand;

PCR/qPCR instruments;

Low-adsorption EP tubes, PCR tubes, and qPCR tubes.

06/Notes

For research only. Not for use in diaognostic procedures.

Due to many factors such as the samples, abundance of the target protein in the sample, and operation, it may be necessary to adjust the experiment procedure as per the actual situation. To obtain high-quality results, please read the following precautions carefully. Contact Vazyme Technical Support for assistance in case of any issues during use: info.biotech@vazyme.com.

06-1/About qPCR Reagents

◇ The 2 × ChamQ Universal SYBR qPCR Master Mix component in the kit is used for qPCR detection of the target genes.

◇ Use of 2 × ChamQ Universal SYBR qPCR Master Mix:

▲ This component contains the fluorescent dye SYBR Green and should be stored away from light. Avoid strong light when preparing the reaction system.

▲ Avoid freezing and thawing this component repeatedly, so as not to cause the decrease of enzyme activity. We recommend storing the component in aliquots if only need a small amount for each reaction.

▲ This product is highly sensitive and susceptible to contamination by airborne aerosols. Therefore, prepare the reaction system on an ultra-clean workbench and use sterile tips, PCR reaction tubes, and qPCR reaction tubes. If conditions permit, use dedicated pipettes and filter tips.

▲ Mix well by inversion before use. Avoid vigorous shaking, which may lead to foaming and affect quantitation results. Avoid vigorous pipetting during sample preparation. If the reagent foams, briefly centrifuge before use.

◇ The component contains a special ROX Passive Reference Dye compatible with all qPCR systems, eliminating the need to adjust ROX concentrations for different instruments.

06-2/About Magnetic Beads

◇ The ConA Beads Pro component in the kit is used to bind cells.

◇ About use of ConA Beads Pro:

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

▲ Magnetic beads should be fully mixed by vortex shaking or pipetting up and down prior to each adsorption.

▲ Do not mix by shaking or pipetting vigorously after ConA Beads Pro bind to cells to avoid the release of cells from the beads.

▲ Avoid prolonged exposure of the beads or bead-cell complexes to air to prevent adverse effects on subsequent tests due to the drying of beads.

▲ Avoid high-speed centrifugation or prolonged attraction of magnetic beads on the magnetic separation rack to prevent bead aggregation.

▲ It is normal that some beads may adhere to the tube wall or aggregate during incubation. As long as the bead-cell complexes are immersed in the solution, the results of subsequent tests will not be affected. The degree of adhesion or aggregation varies across different types of cells that are bound to the beads. For the same type of cells, a larger sample input amount causes more pronounced bead aggregation or adhesion to the tube wall. If beads adhere to the tube wall or aggregate, gently flick the bottom of the tube to mix the bead-cell complexes. Avoid repeated uncapping or mixing by pipetting up and down.

06-3/About Sample Preparation and Antibody Selection

◇ If live cells are used, common suspension cells can be harvested for the assay by centrifugation and removal of medium. For most adherent cells, digest with trypsin, collect the cell suspension, centrifuge, and discard the medium to collect cells. For some cell lines, trypsin digestion may affect the binding of cells to ConA Beads Pro, and the judgment can be performed according to the actual condition.

◇ Cells used in the CUT&RUN experiment can be stained with trypan blue for cell viability assay. The cell viability is preferably >90%. Handle cells as gently as possible during the experiment to maintain cell viability. For cells with poor growth or dead cells, the binding state of protein and DNA will change, and even the protein may detach and become naked DNA. Random nuclease cleavage produces high background noise, severely affecting the experimental results.

◇ For low-abundance target proteins in the sample and certain special transcription factors, light cross-linking of cells may help yield better experimental results.

◇ It is recommended to incorporate positive and negative controls in the experiment. We recommend using high-abundance histones, such as H3K4me3, as a positive control, and IgG as a negative control to identify any potential abnormality throughout the assay. It is not necessary to add non-specific IgG as a negative control as it does not provide valuable information in sequencing analysis. The addition of such control is selected depending on the experimental needs.

◇ We recommend using chromatin immunoprecipitation (ChIP)-grade primary antibodies and IgG for the assay. If there are no commercially available ChIP-grade antibodies against the target protein, you may try using antibodies that are suitable for immune fluorescence (IF) assays.

06-4/About Column-based Extraction Reagents

◇ Before the first use, add the designated volume of ethanol absolute to Buffer GW as indicated on the label of the reagent bottle and mark accordingly. Store at room temperature.

◇ Buffer GDP precipitates when stored at low temperature. Let it stand at room temperature for a while before use, or prewarm it in a 37°C water bath until the precipitate is completely dissolved if necessary, and mix well before use.

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

06-5/About Spike in DNA

◇ The Spike in DNA supplied in this kit is a 300 bp fragment derived from *E. coli* λDNA. It can be used to normalize experimental data acquired under different treatments or cell conditions in quantitative analysis of test results.

◇ Spike in DNA can be diluted with ddH_2O. Dilute low-concentration Spike in DNA right before use and avoid long-term storage at low concentrations. We recommend determining the concentration before dilution and diluting based on the actual concentration to ensure accurate addition of Spike in DNA.

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

ATAACTCAATGTTGGCCTGTATAGCTTCAGTGATTGCGATTCGCCTGTCTCTGCCTAATC
CAAACTCTTTACCCGTCCTTGGGTCCCTGTAGCAGTAATATCCATTGTTTCTTATATAAAG
GTTAGGGGGTAAATCCCGGCGCTCATGACTTCGCCTTCTTCCCATTTCTGATCCTCTTCA
AAAGGCCACCTGTTACTGGTCGATTTAAGTCAACCTTTACCGCTGATTCGTGGAACAGAT
ACTCTCTTCCATCCTTAACCGGAGGTGGGAATATCCTGCATTCCCGAACCCATCGACGA

06-6/Notes on Data Processing

◇ Data processing uses the $2^{-\Delta\Delta C_T}$ algorithm, and the specific processing flow is as follows:

Statistics of the C_T values of the target gene and Spike in DNA in the experimental group, control group, and negative control group;

Using Spike in DNA as the internal reference, calculate the ΔC_T value of each group as follows:

Experimental Group: $\Delta C_{T\ treatment} = C_{T\ treatment} - C_{T\ treatment\ Spike\ in\ DNA}$

Control Group: $\Delta C_{T\ control} = C_{T\ control} - C_{T\ control\ Spike\ in\ DNA}$

Negative Control Group: $\Delta C_{T\ IgG} = C_{T\ IgG} - C_{T\ IgG\ Spike\ in\ DNA}$

Using the negative control IgG as a reference, calculate the $2^{-\Delta\Delta C_T}$ value of each group as follows:

Relative Enrichment $fold_{treatment} = 2^{-(\Delta C_T\ treatment\ -\ \Delta C_T\ IgG)}$

Relative Enrichment $fold_{control} = 2^{-(\Delta C_T\ control\ -\ \Delta C_T\ IgG)}$

Relative Enrichment $fold_{IgG} = 2^{-(\Delta C_T\ IgG\ -\ \Delta C_T\ IgG)} = 1$

06-7/Additional Precautions

◇ Thaw all kit components at appropriate temperature and mix each component thoroughly before use.

◇ Store different buffers and reagents at appropriate conditions to avoid inactivation.

◇ To avoid cross-contamination of samples, it is recommended to use tips with a filter and to replace the tip between different samples.

◇ If handled improperly, PCR products are highly susceptible to aerosol contamination, which affects the accuracy of experimental results. Therefore, it is recommended to physically separate the PCR reaction system preparation area from the PCR product purification and detection areas, use dedicated equipment (e.g., pipettes), and regularly clean each laboratory area with RNase, RNA and DNA Remover (Vazyme #R504) to keep the laboratory environment clean.

◇ Positive Control is a qPCR primer designed specifically for histone H3K4me3 in human cells. It is not applicable to other species.

07/Experiment Process

07-1/Buffer Preparation

▲ The following procedure applies to one sample. Scale the volumes based on the actual number of samples to be tested.

1. Binding Buffer: Dilute 30 µl of 10 × Binding Buffer to 300 µl 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: Dissolve one Protease Inhibitor Cocktail Tablet (Sigma-Aldrich #5056489001) in 1 ml of ddH_2O and 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 pre-cool on ice.
4. Dig-wash Buffer: Add 38 µl of 5% Digitonin to 3.8 ml of Wash Buffer prepared in Step 2 and mix well.
   ▲ Digitonin is toxic. Ensure proper personal protection when preparing the solution. Prepare the buffer right before use because it cannot be stored for a long time after Digitonin is added.
5. Stop Buffer: Add 1 µl of 5% Digitonin to 100 µl of 2 × Stop Buffer(-). Add the appropriate amount of Spike in DNA according to the cell input amount, mix well, and pre-cool on ice.
   ▲ Spike in DNA can be diluted with ddH_2O. It is recommended to dilute the DNA just before use and store at -30 ~ -15°C.
   ▲ Spike in DNA can be used for the normalization of experimental data. For profiling of H3K4me3 in K562 cells, the recommended Spike in DNA input amounts are listed in the following table:

Number of Input Cells	Spike in DNA Input Amount
5,000 - 10,000	1 pg
100,000	10 pg
500,000	50 pg

6. Before the first use, add the designated amount of ethanol absolute to Buffer GW as indicated on the label of the reagent bottle, and mark accordingly.

	Volume of Ethanol Absolute (ml)	
	HD101-01	HD101-02
Buffer GW	20	40

07-2/ConA Beads Pro Treatment

1. Add 100 µl of Binding Buffer to a 1.5 ml EP tube.
2. Resuspend ConA Beads Pro thoroughly by gentle pipetting. Add 10 µl of ConA Beads Pro to the Binding Buffer in Step 1, mix gently, and place the mixture on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant.
3. Remove the EP tube from the magnetic stand, add 100 µl of Binding Buffer, then gently pipette to fully mix (do not mix by vortexing).

4. Place the EP tube on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant, and add 10 µl of Binding Buffer to resuspend the beads.

07-3/Cell Collection

▲ Perform all steps before cell permeabilization at room temperature to minimize the stress on cells. Avoid vigorous vortexing in the procedure.

1. Collect and count cells at room temperature.
2. Transfer the required number of cells to a 1.5 ml EP 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. Add 100 µl of Wash Buffer to each sample to resuspend the cells.

07-4/Cell and ConA Beads Pro Incubation

1. Transfer 100 µl of cells to the EP tube containing treated ConA Beads Pro. After shaking and mixing, incubate at room temperature for 10 min, and shake 2 - 3 times during this period.
   ▲Do not mix by pipetting or vortexing after the cells are added.
2. Briefly centrifuge (< 100 g) to collect the reaction mix. Place the EP tube on the magnetic stand. After the solution becomes clear (about 2 min), discard the supernatant.
   ▲Do not centrifuge for too long, as this may cause the beads to aggregate at the bottom of the tube.

07-5/Primary Antibody Incubation

1. Add 100 µl of pre-cooled Antibody Buffer to each sample to resuspend the cell-magnetic bead complexes.
2. Add the antibody to the EP tube according to the recommended immune concentration in the antibody manual, and invert to mix.
3. Collect the reaction solution at the bottom of the tube with short-spin centrifugation (Do not aggregate the magnetic beads at the bottom of the tube by prolonged centrifugal time), then place the EP tube at 2 ~ 8°C overnight.
   ▲We recommend including a positive control 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 premix. Mix by inversion and place on ice.
   ▲Dilute pG-MNase Enzyme just before use to ensure optimal cleavage activity. To ensure the stability of pG-MNase Enzyme, we recommend aliquoting the component at first use and storing it at -30 ~ -15°C.
2. Briefly centrifuge the EP tube from 07-5/Primary Antibody Incubation to collect the reaction solution. Place the EP tube on the magnetic stand until the solution becomes clear (30 sec - 2 min), and discard the supernatant.

3. Add 800 µl of Dig-wash Buffer to the EP tube and invert several times to ensure that the buffer and cell-magnetic bead complexes are thoroughly mixed.
4. Briefly centrifuge to collect the reaction solution. Place the EP tube on the magnetic stand. After the solution becomes clear (30 sec - 2 min), discard the supernatant.
5. Repeat Steps 3 - 4 (twice in total).
6. Add 100 µl of the pG-MNase Enzyme premix from Step 1 and invert several times to ensure that the buffer and cell-magnetic bead complexes are thoroughly mixed.
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.
2. Briefly centrifuge the EP tube from 07-6/pG-MNase Enzyme Incubation to collect the reaction mix. Place the EP tube on the magnetic separation rack. After the solution becomes clear (30 sec - 2 min), discard the supernatant.
3. Add 800 µl of Dig-wash Buffer to the EP tube and invert several times to ensure that the buffer and cell-bead complex are thoroughly mixed.
4. Briefly centrifuge to collect the reaction mix. Place the EP tube on the magnetic separation rack. After the solution becomes clear (30 sec - 2 min), discard the supernatant.
5. Repeat Steps 3 - 4 (twice in total).
6. Add 100 µl of the $CaCl_2$ premix from Step 1 and invert several times to ensure that the buffer and cell-bead complex are thoroughly mixed.
7. Due to differences in the expression abundance of different target proteins, the corresponding optimal MNase cleavage conditions are different. Researchers can refer to the table below to select the appropriate digestion temperature and digestion time. In addition, the digestion temperature and digestion time can be adjusted according to the actual situation. Over-digestion will cause an increase in background signal.

Target Abundance	Digestion Temperature	Digestion Time
Conventional histone systems such as H3K4me3, etc.	On ice	1 h
Transcription factor systems with high expression abundance such as CTCF, etc.	On ice	1.5 h
Transcription factor systems with low expression abundance such as Bmi1, etc.	4°C	1 - 4 h

07-8/Fragmentation Termination and DNA Fragment Release

1. Add 100 µl of Stop Buffer to the EP tube from 07-7/Fragmentation, and mix well by inversion.
2. Incubate the EP tube in a 37°C water bath for 10 - 30 min.
   ▲ Do not shake the tube in the meantime. Keep the tube still to release the DNA fragments.
3. Centrifuge at 12,900 rpm (16,000 × g) for 5 min at 4°C.
4. Place the EP tube on the magnetic stand. After the solution becomes clear (30 sec - 2 min), transfer the supernatant to a new 1.5 ml EP tube.
   ▲ The supernatant from Step 4 contains chromatin-enriched products, which 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 EP tube from 07-8/Fragmentation Termination and DNA Fragment Release of DNA Fragments, mix well by vortexing, and incubate at room temperature for 10 min with mixing 2 - 3 times by inversion.
2. Briefly centrifuge to collect the droplets from the tube wall. Place a FastPure gDNA Mini Columns in a Collection Tube 2 ml. Transfer 650 µl of the sample solution to the spin column, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
3. Discard the flow-through and place the spin tube in the Collection Tube. Transfer the rest of the sample solution to the spin tube, and centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
4. Discard the flow-through and place the spin tube in the Collection Tube. Add 700 µl of Buffer GW (supplemented with absolute ethanol) to the spin tube. Centrifuge at 12,000 rpm (13,400 × g) for 60 sec.
   ▲ Add Buffer GW along the wall of the spin column, or cap the column and invert 2 - 3 times after adding Buffer GW to fully rinse the residual liquid on the column wall.
5. Discard the flow-through and place the spin tube in the Collection Tube. Centrifuge at 12,000 rpm (13,400 × g) for 2 min.
6. Leave the spin tube uncapped at room temperature for 2 - 5 min.
   ▲ Air dry the adsorptive membrane thoroughly to ensure DNA purity.
7. Place the spin tube in a new 1.5 ml EP tube. Add 20 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 tube and store the extracted product at -30 ~ -15°C.
   ▲ The product from Step 8 contains the target gene fragment, which can be stored at -30 ~ -15°C for 7 days.

07-10/qPCR

1. Prepare a mixture with the following components in a qPCR tube:

Components	Volume
2 × ChamQ Universal SYBR qPCR Master Mix	10 μl
Primer F (10 μM)	0.5 μl
Primer R (10 μM)	0.5 μl
Extracted DNA	2 μl
RNase-free ddH_2O	7 μl
In total	20 μl

The amount of each component in the reaction system may be adjusted according to the following principles:

- ▲ For low-abundance target proteins, the input amount of extracted DNA may be increased as appropriate to 2 - 9 μl. Make sure that the input volumes of extracted DNA in the experimental and control groups are the same.
- ▲ In general, a final primer concentration of 0.2 μM in the reaction system yields good amplification results. In case of poor performance, adjust the primer concentration within a final concentration range of 0.1 - 1.0 μM.
- ▲ qPCR is extremely sensitive. Therefore, the accuracy of the amount of templates added to the reaction system can greatly affect the final quantification results. Add the accurate volume of templates and ensure that all the liquid in the pipette tip is transferred into the reaction system.

2. Place the PCR tube into the qPCR instrument and run the following program:

Stage 1	Initial Denaturation	Rep: 1	95°C	30 sec[a]
Stage 2	Cycling Reaction	Reps: 40	95°C	10 sec
			60°C	30 sec
Stage 3	Melt curve[b]	Rep: 1	95°C	15 sec
			60°C	60 sec
			95°C	15 sec

a. Most templates can be amplified under this initial denaturation condition. For templates with complex structures, the initial denaturation time may be extended to 3 min to obtain better results.

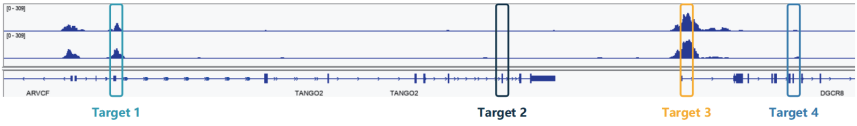
b. Melt curve programs vary across qPCR instruments. Use the default of your qPCR system.

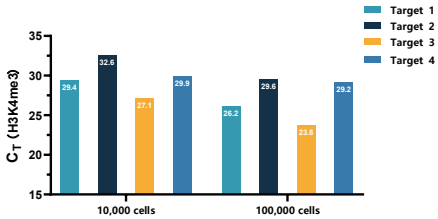
07-11/qPCR Result Analysis

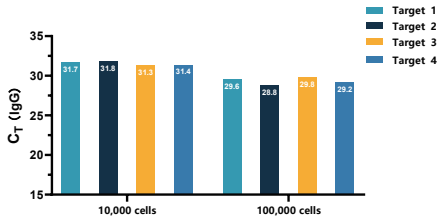
1. C_T value, $-\Delta\Delta C_T$ value and Relative Enrichment fold:

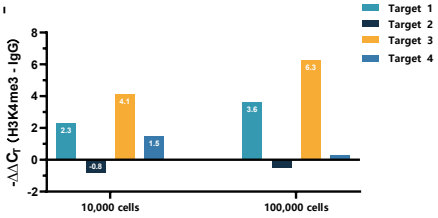
Take the CUT&RUN-qPCR experiment of histone H3K4me3 and H3K27me3 using 10,000 and 100,000 HeLa cells as an example. Select different gene sites from their respective IGVs to design primers, and use these primers and Spike in DNA primers to detect the relative enrichment of the target gene. The results are shown in the figure below.

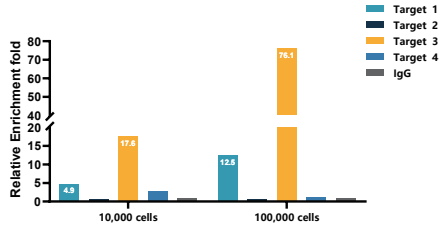
Hela, H3K4me3 chr22:20,010,059-20,129,589



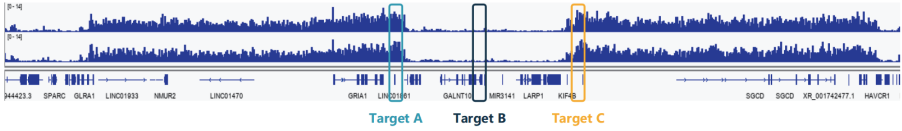


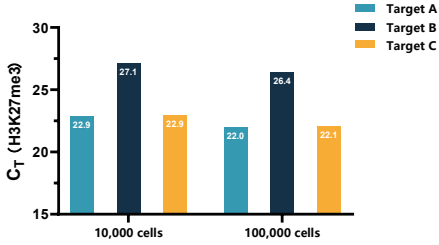


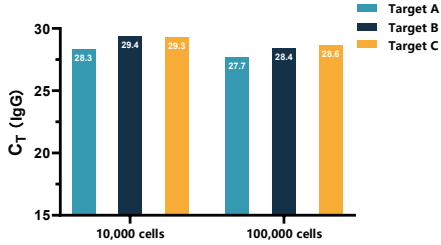


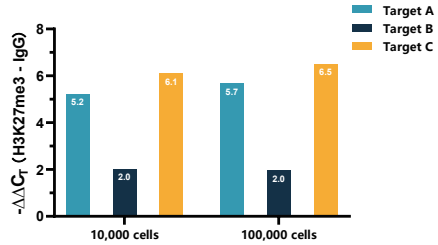


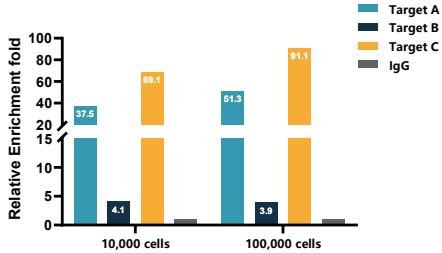
Hela, H3K27me3 chr5:151,348,209-158,180,205







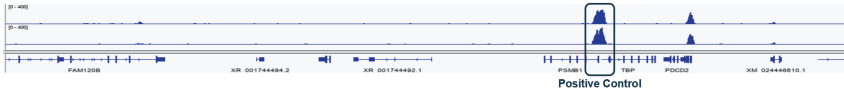


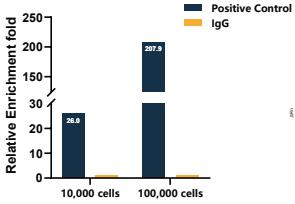


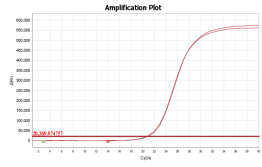
2. Example of a positive control:

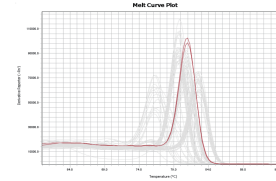
Take 100,000 Hela cells to perform a CUT&RUN-qPCR experiment on histone H3K4me3 as an example. Take 2 μl of the extracted product as a template, and use the positive control primer to detect the relative enrichment of the TBP gene. The results are as shown in the figure below:

Hela, H3K4me3 chr6:170,353,473-170,755,228









08/FAQ & Troubleshooting

◇What species can CUT&RUN be applied to?

The CUT&RUN protocol is widely applicable for studying protein-DNA interactions in common mammalian cells. Yeast and plant cells should be treated before proceeding with the assay, but low cell input is not recommended as this may lead to poor results.

◇What are ConA magnetic beads mainly used for?

Concanavalin A (ConA)-coated magnetic beads bind to glycoproteins on the cell membrane, thereby adsorbing cells, visualizing cell processing operations, and reducing cell loss during the subsequent experiments.

◇Primer Design Guidance of CUT&RUN-qPCR:

1. Amplified fragment size: Recommend 80 - 200 bp, 80 - 150 bp is optimal.
2. Primer length: Recommend 18 - 25 nt, and the difference between the upstream and downstream primers should be no more than 3 nt.
3. Tm value: The difference between the Tm values of the forward and reverse primers should be no more than 1°C, and the primer Tm should be adjusted close to 60°C.
4. GC content: Recommend 40% - 60%, 4 types of bases are evenly distributed in the primer.
5. The 3' end should try to avoid areas with high GC or high AT content.
6. The last base at the 3' end should be G or C, avoid using T.
7. Use NCBI BLAST to check the specificity of primers; Database recommends selecting Refseq representative genomes.

Nanjing Vazyme Biotech Co.,Ltd.
Tel: +86 25-83772625
Email: info.biotech@vazyme.com
Web: www.vazyme.com
Loc: Red Maple Hi-tech Industry Park, Nanjing, PRC
Follow Us