CUT&Tag Assay Kit (pAG-Tn5) for illumina
CSB-DKT072
Product Manual

Product Description

The CUT&Tag Assay Kit for Illumina is a kit specifically developed for the study of protein-DNA interactions based on Illumina's high-throughput sequencing platform. The Cleavage Under Target & Tagmentation (Cleavage Under Target & Tagmentation) technology is A new approach to protein-DNA interactions. It uses Protein A/G fused transposase to target target proteins under the guidance of antibodies and cut DNA near the target site. At the same time, the necessary splicing sequence was introduced, the product DNA was extracted and amplified by PCR, and the library was obtained directly for sequencing. Compared with the traditional CHE-SEQ, this kit has the advantages of low cell input, short experimental period, high signal-to-noise ratio and good repeatability. It is especially suitable for the research fields of early embryonic development, stem cells, tumor and epigenetics. The reagents in the kit have undergone strict quality control to ensure the stability and repeatability of the library construction to the greatest extent.

Product composition and storage conditions

	Component	4T	12T	storage conditions
Box Ⅰ	pAG-Tn5 Transposome(4uM)	4 μL	12 μL	Store at -20℃
	5% Digitonin*	64 μL	192 μL	
	1 M MgCl2	10 μL	30 μL	
	10×ConA Binding Buffer	120 μL	360 μL	
	10×Wash Buffer	1 mL	1.5 mL*2	
	10×Dig-300 Buffer	500 μL	1.5 mL	
	Antibody Buffer（-）	300 μL	900 μL	
	Proteinase K(20 mg/mL)	4 μL	12 μL	
	5×Stop Buffer	100 μL	300 μL	
	2×PCR Mix for NGS	150 μL	450 μL	

***Note**: Digitonin has moderate toxicity and should avoid direct contact with the skin; Ice bath will cause it to solidify, and during the experiment, it will be placed at room temperature. After adding Digitonin, the buffer solution cannot be stored for a long time, and is now ready for use.

	Component	4T	12T	storage conditions

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Box II	ConA Beads	40 μL	120 μL	Store at 2-4℃ to avoid repeated freezing
	DNA Clean Beads	750 μL	1.2 mL*2	

Self prepared materials

Antibodies (primary and secondary antibodies), protease inhibitors (50 ×);

Other materials: anhydrous ethanol, sterilized ultrapure water, low adsorption EP tube, PCR tube, rotary mixer, magnetic rack, PCR instrument, etc

Points For Attention

1. Sample preparation

1.1 Before the formal experiment, in order to reduce the risk of experimental failure, please test the binding efficiency between cells and ConA magnetic beads to ensure a high binding rate before starting the experiment.

1.2 If live cells are used for the experiment, for common suspension cell lines, the upper layer of culture medium can be discarded after centrifugation to collect cells for the experiment. For most adherent cells, after digestion with trypsin, collect the cell suspension, centrifuge, and discard the upper culture medium to collect the cells. For some cell lines, the use of trypsin digestion may affect the binding effect between cells and ConA Beads, and judgment needs to be made based on the actual situation.

1.3 Cells used for CUT&Tag experiments can be stained with trypan blue for cell viability testing, with activity preferably>90%. During the experiment, the manipulation of cells should be as gentle as possible to maintain cell viability. In cells with poor growth or death, the binding state between proteins and DNA can change, even causing protein detachment and becoming exposed DNA. Random cleavage of transposons may generate strong background noise, which can affect experimental results.

1.4 Using nuclear cells for experiments requires exploring suitable conditions for nuclear extraction based on different tissue types.

2. Antibody selection

2.1 It is recommended to set up a positive control group and a negative control group in the experiment. Histone proteins with high expression abundance in the samples are recommended for the positive control group, and an experimental group with normal addition of secondary antibodies and transposons without adding primary antibodies is recommended for the negative control group to judge whether there are abnormalities in the whole experiment process. It is not necessary to add non-specific IgG negative control group. It does not provide valuable information in the sequencing analysis, so you can choose whether to add it according to the needs of the experiment.

2.2 In the experiment, it is recommended to use ChIP level antibodies. IF no ChIP level antibodies are available for interest protein, antibodies suitable for IF experiment can be used for testing.

2.3 Be sure to select secondary antibodies with high Protein A/G affinity and without modification.

3. Use of magnetic beads

3.1 ConA Beads and DNA Extract Beads should be stored under conditions of 2-8 ℃, and should not be stored below 0 ℃.

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3.2 Magnetic beads should be balanced to room temperature before use, and all magnetic bead operations should be carried out at room temperature.

3.3 Before each suction of the magnetic beads, they should be thoroughly vortex mixed or blown up and down using a pipette to thoroughly mix.

3.4 After ConA Beads bind to cells, do not vortex shake or vigorously blow with a pipette. Gently suck and beat to avoid reducing the efficiency of binding cells.

3.5 Do not expose ConA Beads or ConA Beads cell conjugates to air for extended periods of time, as drying of the magnetic beads will affect subsequent experiments.

3.6 Do not centrifuge ConA Beads or ConA Beads cell conjugates at high speed or place them on a magnetic shelf for a long time to avoid accumulation of magnetic beads.

3.7 It is normal for some magnetic beads to aggregate or adhere to the wall during the incubation process, as long as the ConA Beads cell binding body is within the solution infiltration range.

3.8 When aspirating the supernatant, do not aspirate onto the magnetic beads. The magnetic beads should avoid excessive drying.

4. Cell input and amplification cycles

4.1 The suitable amount of cell input for the kit is 60-100,000 cells. The minimum compatible amount of cell input is not fixed depending on cell type, antibody selection, and abundance of interest protein expression. 10,000-50,000 cells are recommended for early experiments.

4.2 The small amount of cells required for CUT&Tag experiment provides the possibility for extremely low cell initiation and single-cell experiment, but it is not recommended to use too low cell initiation for CUT&Tag experiment. If single-cell experiment is conducted, the experimental process should be optimized in order to obtain better experimental results.

4.3 When PCR amplification is performed, the output of the library can only meet the requirements of the computer. Usually, the 20ng library distributed in the range of 200bp to 1000bp has met the requirements of the computer, and there is no need to set too high the number of amplification cycles in order to obtain higher library output. Excessive number of cycles can lead to excessive expansion, increased preference, increased repetition and other adverse consequences.

5. Detection of library length distribution

The length distribution of the library can be detected using devices based on electrophoretic separation principles such as LabChip GX, GXII, GX Touch (PerkinElmer), Bioanalyzer, Tapestation (Agile Technologies), Fragment Analyzer (Advanced Analytical), etc.

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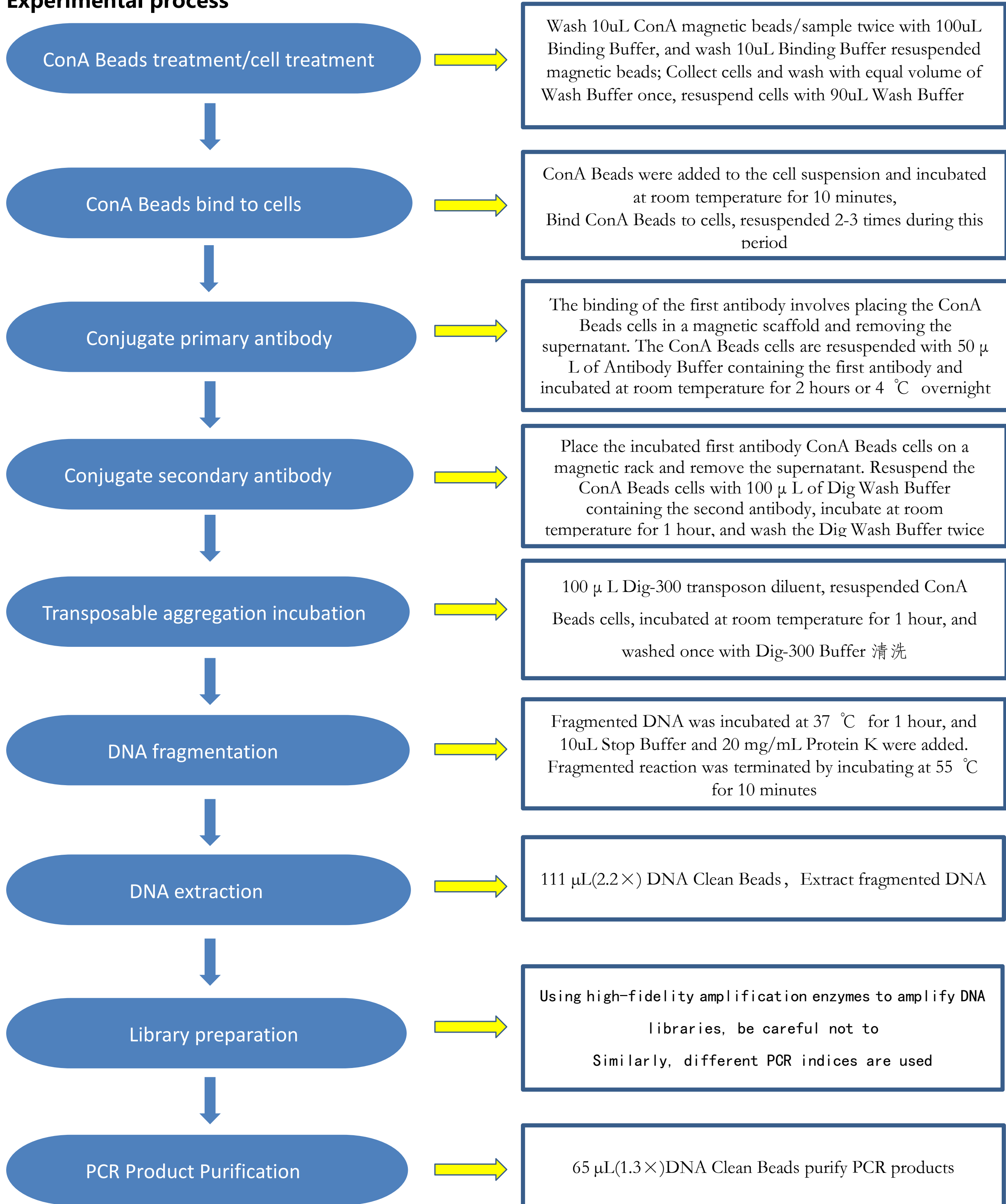
Experimental process



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Operating Steps

1. Reagent preparation

(The reagent dosage is calculated based on a single sample here, please prepare in proportion to the actual sample quantity)

1.1 Binding Buffer: Take 30 μ L 10 × Binding Buffer, add ddH2O to 300 μ L, and mix well.

1.2 Water Buffer: Take 200 μ L 10 × Wash Buffer, add 40 μ L protease inhibitor (50 ×), Add ddH2O to 2 mL and mix well.

1.3 Anti body Buffer: Take 50 μ L Anti body Buffer (-), add 0.5 μ L 5% Digitonin, mix well, and place on ice for pre cooling.

1.4 Dig water Buffer: Take 792 μ L of the water buffer prepared in step 2, add 8 μ L of 5% Digitonin, and mix well.

1.5 Dig-300 Buffer: Take 100 μ L 10 × Dig-300 Buffer, with 2 μ L of 5% Digitonin and 20 μ L of protease inhibitor (50 ×), Add 878 μ L ddH2O and mix well.

2. ConA Beads processing

2.1 Take out ConA Beads from a 4 ℃ refrigerator, balance at room temperature, blow and mix. Take 10 μ L magnetic beads and place them in a 1.5 mL EP tube. Place the 1.5 mL EP tube on a magnetic rack and let it stand for 2 minutes until the solution becomes clear. Discard the supernatant.

2.2 Add 100 μ L Binding Buffer resuspended magnetic beads to a 1.5 mL EP tube, gently blow and mix with a pipette, then place on a magnetic rack and let stand for 2 minutes. Wait for the solution to clear, and discard the supernatant.

2.3 Remove the EP tube from the magnetic bracket and repeat step 2.2 once.

2.4 Add 10 μ L Binding Buffer resuspended ConA Beads to the EP tube, blow and mix well, and place at room temperature for later use (if the cell treatment time is too long, place in an ice bath).

3. Cell processing

3.1 Collect cells at room temperature and count them. Take the required number of cells for the experiment and place them in a 1.5 mL EP tube (collect cells with a volume not exceeding 1 mL) at room temperature of 600 × Centrifuge at low speed for 5 minutes and carefully discard the supernatant.

3.2 Add a Wash Buffer of the same volume as the initial cell suspension at room temperature and gently blow the resuspended cells. Centrifuge 600g at room temperature for 5 minutes, and carefully discard the supernatant.

3.3 Add 90 μ L Wash Buffer to a 1.5 mL EP tube and gently blow the resuspended cells.

4. Binding of cells to magnetic beads

Transfer 90 μ L cells to EP tubes of activated ConA Beads, gently blow 5-10 times, mix well, and incubate at room temperature for 10 minutes. During this period, resuspend 2-3 times (or place in a shaker/rotary mixer at 100 rpm/min).

5. Primary antibody incubation

5.1 Dilute the first antibody into the prepared Antibody Buffer, and each sample requires 50 μ L of Antibody Buffer. The dilution ratio is adjusted according to the specific situation of the antibody, and we recommend a dilution volume ratio of 1:50-1:100 for the first antibody. Dilute the first antibody, blow and mix well, and precool on ice.

5.2 Gently and instantly separate the incubated ConA Beads cells and place them on a magnetic rack for 2 minutes. After the liquid clears, carefully discard the supernatant. Remove the EP tube from the magnetic holder, add 50 μ L pre cooled Antibody Buffer with added first antibody, and gently blow 5-10 times to mix well.

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5.3 Immediately transfer the mixed sample to a rotary mixer and incubate at room temperature for 2 hours, 100 rpm/min, or 4 ℃ overnight.

6. Secondary antibody incubation

6.1 Dilute the secondary antibody with a Dig-wash Buffer in a certain proportion (it is recommended to use a 1:100 dilution as a routine), with 100 μ L of each sample (prepared before use).
6.2 Collect the reaction solution from the EP tube incubated with the first antibody by instantaneous centrifugation, place it on a magnetic rack, wait for the solution to clear for 2 minutes, and discard the supernatant completely.
6.3 Remove the EP tube from the magnetic holder, add 100 μ L of Dig-wash Buffer with added secondary antibody, resuspend all ConA Beads cells, gently blow and mix, and immediately transfer the mixed sample to a rotary mixer. Incubate at room temperature for 1 hour.
6.4 Remove the EP tube that has incubated the secondary antibody, collect the reaction solution by instantaneous centrifugation, place the EP tube on a magnetic rack, wait for the solution to clear for 2 minutes, and carefully discard the supernatant.
6.5 Remove the EP tube, add 200 μ L Dig-wash Buffer, invert it several times to ensure that the buffer is fully mixed with the cell (nucleus) magnetic bead complex, and let it stand at room temperature for 5 minutes.
6.6 Repeat steps 6.4 to 6.5 .

7. Assembly and cleaning of the swivel body

7.1 Dig-300 transposable diluent: Dilute 1 μ L pAG-Tn5 transposome into 100 μ L Dig-300 Buffer, blow and mix well, with a dosage of 100 μ L for each sample.
7.2 Place the sample after standing in step 6.6 on a magnetic rack and let it stand for 2 minutes. After the liquid has clarified, carefully discard the supernatant.
7.3 Remove the EP tube from the magnetic holder, add 100 μ L Dig-300 transposable diluent, resuspend ConA Beads cells, gently blow and mix;
7.4 Immediately place the mixed sample on a rotary mixer and incubate at room temperature for 1 hour.
7.5 Remove the EP tube that incubates the improved seat and gently detach it. Place it on a magnetic rack and let it stand for 2 minutes. After the liquid clears, carefully discard the supernatant.
7.6 Remove the EP tube from the magnetic holder, add 200 μ L Dig-300 Buffer to each tube of sample, gently blow and mix with a pipette, resuspend all ConA Beads cells, and let stand at room temperature for 5 minutes.
7.7 Repeat steps 7.5-7.6 .

8. DNA Fragmentation

8.1 Composition Buffer Preparation: Take 50 μ L of Dig-300 Buffer, add 0.5 μ L of 1 M MgCl2, vortex mix well, and prepare each sample at 50 μ L.
8.2 Instantly centrifuge the sample in 7.7 to collect the reaction solution, place it on a magnetic rack and let it stand for 2 minutes. After the liquid clears, carefully discard the supernatant.
8.3 Remove the EP tube from the magnetic holder, add 40 μ L Tagmentation Buffer, resuspend ConA Beads cells, gently blow and mix.
8.4 Immediately place the mixed sample in a shaker at 100 rpm/min and incubate at 37 ℃ for 1 hour.

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8.5 Take out the incubated sample and gently separate it. Add 10 μ L of 5 to each sample tube × Stop Buffer, vortex mix well and add 0.5 μ L Protein K; After brief centrifugation, incubate in a metal or water bath at 55 ℃ for 10 minutes to terminate the fragmentation reaction.

9. DNA extraction

9.1 Take out the DNA Extract Beads from a 4 ℃ refrigerator in advance, balance at room temperature for 30 minutes, and mix the magnetic beads with vortex oscillation for later use.

9.2 Adding 111 to the fragmented sample μ L (2.2 ×) DNA Clean Beads, thoroughly vortex mix, and incubate at room temperature for 5 minutes.

9.3 Move the EP tube to the magnetic support and let it stand still. After the liquid clears, carefully discard the supernatant.

9.4 Place the EP tube on a magnetic rack, add 200 μ L of freshly prepared 80% ethanol to rinse the magnetic beads, let it stand at room temperature for 30 seconds, and carefully discard the supernatant.

9.5 Repeat step 9.4 once, after instant separation, move the EP tube to the magnetic support and let it stand. After the magnetic beads are separated from the solution, use a 10 μ L gun head to thoroughly absorb the residual liquid9.6 Dry at room temperature for 3-5 minutes to allow the ethanol to fully evaporate. Remember not to excessively dry the magnetic beads.

Note: The drying time may vary depending on the temperature and humidity of the environment. Judgment criteria: The surface of the magnetic beads changes from moist bright to dull, 50% of the magnetic beads change from brown yellow to dry yellow, and there is no obvious liquid residue on the tube wall.

9.7 Remove the EP tube from the magnetic holder, add 22 μ L of sterilized ultrapure water to each tube, blow and mix with a pipette, elute at room temperature for 5 minutes, and gently shake 2-3 times during this time.

9.8 Centrifuge the EP tube briefly and place it on a magnetic rack. Wait for the solution to clear, carefully suck 20 μ L of supernatant into a new sterile EP tube. The samples are stored at -20 ℃ or directly subjected to the next step of PCR amplification.

10. Library amplification

10.1 Prepare the following components in a sterile PCR tube:

Component	Volume
Fragmented DNA	20 μL
2×PCR Mix for NGS	25 μL
N5 Primer（10μM）	2.5 μL
N7 Primer（10μM）	2.5 μL
Total	50 μL

10.2 Use a pipette to gently blow 10 times and mix thoroughly. Perform the following reaction in the PCR instrument:

Temperature	Time	Cycles
72°C	3 min	1
98°C	30 s	1
98°C	15 s	X^b

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60°C	10 s	
72°C	10 s	
72°C	1 min	1
4°C	Hold	---

Note: b. The number of amplification cycles can be adjusted based on the initial DNA input and experimental needs.

11. Purification of PCR products

11.1 Vortex Oscillation Mix DNA Clean Beads and Suck 65 μ L (1.3 ×) In the PCR reaction products mentioned above, vortex oscillation or use a pipette to blow 10 times to ensure the uniformity of the entire system, and incubate at room temperature for 5 minutes.

11.2 Centrifuge the reaction tube briefly and place it on a magnetic rack to separate the magnetic beads and liquid. After the solution has clarified (about 5 minutes), carefully remove the supernatant and avoid disturbing the magnetic beads.

11.3 Keep the PCR tube always on the magnetic rack and add 200 μ Rinse the magnetic beads with freshly prepared 80% ethanol and incubate at room temperature for 30 seconds. Carefully remove the supernatant.

11.4 Repeat step 11.3.

11.5 Keep the PCR tube always on the magnetic rack and open the lid and air dry for 3-5 minutes.

11.6 After drying the magnetic beads, remove the PCR tube from the magnetic rack and add 22 μ L sterilized ultra pure water elution, vortex oscillation or use a pipette to blow 10 times to thoroughly mix the magnetic beads, and incubate at room temperature for 5 minutes.

11.7 Centrifuge the PCR tube briefly, place it on a magnetic rack to separate the magnetic beads and liquid, wait for the solution to clear (about 5 minutes), and carefully suck out 20 μ Transfer the L supernatant to a new EP tube and store at -20 ℃.

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Appendix

1. Transposable joint sequence

ME: 5 '- [phos] CTGTCTTATACACATCT-NH2-3'

P1: 5 '- TCGTCGGGCAGCGTCAGATGTGTATAAGAGAGAG-3'

P2: 5 '- GTCTCGGGGCTCGGAGATGTATAAGAGAGAGAGAGAGAG-3'

2. Library structure

5 '- ATGATACGGCGGACCACCGGATCTACAC-Index2 (i5) - TCGTCGGCAGCGTCAGATGTGTGTAAGA GACAG-xxxxxxxxx-CTGTCTATACACTCCGAGCCCACGACGAC-Index1 (i7) - ATCCGTATGCCGTCTTCTGCTTG-3 ', where - xxxxxxxxx - is an insertion sequence and both ends are library structures.

3. Regarding cell input and amplification cycles

Taking K562 cells as an example, CUT&Tag experiments were conducted using histones with medium to high abundance expression, such as H3K4me3 and H3K27me3,The relationship between input, amplification cycles, and library yield is shown in the table below:

Number of cell input	amplification cycles
100	19 - 21
1,000	16 - 18
10,000	13 - 15
100,000	10 - 12

The small amount of cells required for CUT&Tag experiments provides the possibility for low cell initiation and single cell experiments. However, it is not recommended to use low cell initiation for CUT&Tag experiments. If conducting single cell experiments, in order to obtain ideal experimental results, it is necessary to optimize the experimental process.

When conducting PCR amplification, the output of the library only needs to meet the needs of the computer. Usually, a library with a concentration of 1-3nmol meets the experimental requirements, and there is no need to set an excessively high amplification cycle number to achieve higher library output. Excessive number of cycles can lead to multiple negative consequences such as excessive amplification, increased preference, and increased repeatability.

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