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We use this protocol and it's working

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CUT & RUN

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ABSTRACT

CUT & RUN protocol

1 EpiCypher CUTANATM ChIC/CUT&RUN kit (Kit Version 2.0, User Manual Version 2.1) Catalog No. 14-1048, 48 ChIC/CUT&RUN samples

Overview of the CUTANA CUT&RUN protocol

1. Immobilize & permeabilize cells (or nuclei)
2. Add antibody to histone PTM or chromatin-interacting protein
3. Add & activate pAG-MNase to cleave target-DNA complex
4. Target-DNA complex diffuses out, collect supernatant
5. Extract DNA & prepare sequencing library
6. Next-generation sequencing and data analysis

BOX 1 (at RT under the PCR machine): DNA Binding Buffer (25 mL), DNA Wash Buffer (5 mL), EDTA 100 mM (500 uL), $CaCl_2$(500 uL), DNA Elution Buffer (1.2 mL), DNA Cleanup Columns (50 Columns), DNA Collection Tubes (50 Tubes), 8-Strip Tubes (56 Tubes)

BOX 2 (at 4°C under the PCR machine): SA Beads (40 uL) (X), ConA Beads (550 uL) -> (Jan 2023 Re-ordering), Stop Buffer (1.5 mL), Bead Activation Buffer (12 mL), Pre-Wash Buffer (105 mL)

BOX 3 (at-20 °C in Franklin(Nicole's)): IgG Negative Control (10 uL), H3K4me3 Positive Control (10 uL), Spermidine (100 uL), pAG-MNase (48 runs), Spike-in DNA (100 ng), 5% Digitonin (100 uL)

+ EpiCypher 50 uL - 0.2 mL Magnetic Rack, Invivogen 1.5 mL tube Magnetic Rack

Day 1

Jan 14, 2023

1. Buffer preparation: Make CUT&RUN buffers fresh the day of use.

1) Add 1.8 mL Pre-Wash Buffer per sample to a 50 mL conical tube labeled "Wash Buffer".
2) Dissolve 1 protease inhibitor tablet (Roche) in 2 mL water (25X stock). Add 72 uL per sample to the Wash Buffer. Store remaining 25X stock for 12 weeks at -20°C.
3) Dilute 1M Spermidine 1:2,000 in the Wash Buffer. Store final buffer at RT.
4) Transfer 1.4 mL of Wash Buffer per sample into a new 50 mL conical tube labeled "Cell Perm Buffer". Add 5% Digitonin (1:500 dilution).
5) Transfer 100 uL per sample of Cell Permeabilization Buffer into a new 50 mL tube labeled "Antibody Buffer". Add 0.5 M EDTA (1:250 dilution). Store final buffer on ice.
6) Store the remaining Cell Perm Buffer at 4°C overnight (for Day 2 use).

A.**Wash buffer**: Leave at RT for use on Day 1
Per sample: 1.8 mL Pre-Wash Buffer, 72 uL Protease inhibitor (1X final), 0.9 uL Spermidine (0.5 mM final)
B.**Cell Permeabilization Buffer**: Store at 4°C overnight for use on Day 2
Per sample: 1.4 mL Wash Buffer, 2.8 uL Digitonin (0.01% final)
C.**Antibody Buffer**: Set on ice for use on Day 1
Per sample: 100 ul Cell Perm. Buffer, 0.4 uL EDTA (2 mM final)

2. Bead activation

1) Gently resuspend the**ConA Beads**by pipetting.Transfer 11 uL/sample to a 1.5 mL tube for batch processing.
6 samples: Total 66 ul
2) Place the tube on a magnet until slurry clears and pipette to remove sup.
3) To avoid drying the beads, immediately add 100 uL/sample cold**Bead Activation Buffer**. Pipette gently to mix.
4) Place the tube on a magnet until slurry clears and pipette to remove sup. Repeat previous step for total of two washes.
5) Resuspend beads in 11 uL/sample cold**Bead Activation Buffer**.
6) Aliquot 10 uL/sample of activated bead slurry into separate 8-strip tubes. Keep on ice until needed.
7) Harvest 0.5 million cells/sample in 1.5 mL tube. Centrifuge at 600 x g, 3 min at RT. Decant or pipette culture media sup.
a) 0.5 M: FLAG, b) 1.0 M: FLAG, c) 0.5 M: V5, d) 1.0 M: V5, e) 0.5 M: IgG negative control, f) 0.5 M: H3K4me3 positive control
8) Resuspend cells in 100 uL/sample RT**Wash Buffer**. Pipette to thoroughly resuspend.Centrifuge at 600 x g, 3 min at RT. Decant or pipette sup.
9) Repeat previous step for total of two washes.
10) Resuspend cells in 105 uL/sample in RT**Wash Buffer**and thoroughly pipette to mix. Aliquot 100 uL washed cells to each 8-strip tube containing 10 uL of activated beads. Gently vortex and/or pipette until evenly resuspended.
11) Incubate cell-bead slurry on benchtop for 10 min at RT to adsorb cells to beads.

3. Antibody binding

12) If using a multi-channel pipette (recommended) place a multi-channel reagent reservoir on ice.
13) Place the 8-strip tubes on an 8-strip magnet (high volume setting) until slurry clears. Pipette to remove sup, taking care to avoid disturbing beads with tip.
14) Immediately add 50 uL cold**Antibody Buffer**to each sample and gently vortex and/or pipette mix to prevent beads from drying.
15) Add 2 uL CUTANA H3K4 MetStat Spike-in Control dNucs to the cell samples designated for

the positive (H3K4me3) and negative (IgG) control antibodies.
16) Add 0.5 ug antibody to each sample and gently vortex.
*** Control antibodies included in this kit are provided at 0.5 mg/mL, therefore add 1.0 uL per sample.
FLAG antibody: 1 mg/mL -> 0.5 uL
V5 antibody: 1 mg/mL -> 0.5 uL
IgG, H3K4me3 antibodies: 1.0 uL
17) Incubate 8-strip tubes on nutator (capped ends elevated) overnight at 4°C
18) Store the**Cell Permeabilization Buffer**at 4°C overnight for use on Day 2.

Day 2
Jan 15, 2023
4. Antibody binding, continued
19)If using a multi-channel pipette (recommended) place a multi-channel reagent reservoir on ice.Fill with**Cell Permeabilization Buffer**.
20) Place the 8-strip tubes on magnet until slurry clears. Pipette to remove sup.
21)While beads are on magnet, add 200 uL cold**Cell permeabilization Buffer**directly onto beads. Pipette to remove sup.
22) Repeat previous step for total of two washes, without removing 8-strip tubes from the magnet.
23) Add 50 uL cold**Cell Permeabilization Buffer**to each sample. Gently vortex and/or disperse clumps by thorough pipetting.
Permeabilization: the puncturing of the cell membrane

5. Binding of pAG-MNase
pAG-MNase enzyme is a fusion of protein A and protein G to micrococcal nuclease.
24) Add 2.5 uL pAG-MNase (20X stock) to each sample. Gently vortex/pipette mix.
25) Incubate samples for 10 min at RT. Return 8-strip tube to magnet. Remove sup.
26)While beads are on magnet,add 200 uL cold **Cell permeabilization Buffer** directly onto beads. Pipette to remove sup.
27)Repeat previous step for total of two washes, without removing 8-strip tubes from the magnet.
28) Remove 8-strip tubes from the magnet. Add 50 uL cold**Cell permeabilization Buffer**to each sample. Gently vortex and/or disperse clumps by thorough pipetting.

6. Targeted chromatin digestion and release
29) Place 8-strip tubes on ice. Add 1 uL**100 mM Calcium Chloride**to each sample and gently vortex. Ensure efficient digestion by making sure beads are thoroughly resuspended. Gently pipette with a P200 if needed.
30) Incubate 8-strip tubes on nutator for 2 hours at 4°C

31) Add 33 uL **Stop Buffer** to each sample. Gently vortex to mix.
32) Prior to first use, reconstitute***E.coli*Spike-in DNA** in 200 uL DNase free water.
33) Add 1 uL (0.5 ng) **Spike-in DNA** to each sample. Gently vortex to mix.
34) Incubate 8-strip tubes for 10 min at 37°C in a thermocycler. (Setting: Incubate mode)
35) Quick spin in benchtop microfuge.
36) Place 8-strip tubes on a magnet stand until slurry clears. Transfer sups containing CUT&RUN enriched DNA to 1.5 mL tubes and discard ConA Beads.

7. DNA purification
37) Add 420 uL **DNA Binding Buffer** to each sample. Mix well by vortexing.
38) For every sample, place a **DNA Cleanup Column** into a **DNA Collection Tube**. Load each sample onto a column and label the top.
39) Centrifuge at 16,000 x g, 30 sec, RT. Discard the flow-through. Place the column back into the collection tube.
40) Prior to first use, add 20 mL > 95% ethanol to**DNA Wash Buffer**.

8. DNA purification, continued
41) Add 200 uL **DNA Wash Buffer** to each sample column.
42)Centrifuge at 16,000 x g, 30 sec, RT. Discard the flow-through. Place the column back into the collection tube.
43) Repeat for a total of two washes.
44)Discard the flow-through.Centrifuge one additional time at16,000 x g, 30 sec to completely dry the column (Empty spin).

45) Carefully remove the column from the collection tube, ensuring it does not come into contact with the flow-through. Transfer column to a clean pre-labeled 1.5 mL microfuge tube.
46) Elute DNA by adding 12 uL**DNA Elution Buffer**, taking care to ensure the buffer is added to the center of the column rather than the wall. Tap the column + microfuge collection tube on the benchtop to ensure all droplets are absorbed onto the resin.
*** 12 uL is recommended, however DNA can be eluted in 6-20 uL volumes depending on anticipated yield and desired final concentration. Larger elution volumes, longer incubation times, and/or multiple rounds of elution may improve DNA yield. However, sample concentration will be reduced with larger total elution volume.
47) Let sit 5 minutes, then centrifuge at16,000 x g, 1 min, RT.
48) Vortex eluted material and use 1 uL to quantify the CUT&RUN-enriched DNA using the Qubit fluorometer as per the manufacturer's instructions. See Quality Control Checks section for typical DNA yields.
49) CUT&RUN DNA can be stored at -20°C for future processing.

Measure the DNA concentration using Nanodrop

Blank: DNA Elution Buffer 1ul

Day 3

Jan , 2023

9. NGS library preparation