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CUT and RUN - Cas9 crosslinked

Forked from CUT and RUN

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anita.adami[1]

[1]Laboratory of Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, BMC A11, Lund University, 221 84 Lund, Sweden.

Anita Adami
Lund University

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We use this protocol and it's working

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Abstract

This protocols describe how to perform crosslink CUT&RUN on fresh cells

Sample preparation

25m

1 Detach cultured cells with Accutase (37 °C incubation for 5 minutes, or until cells completely detach) and resuspend in washing buffer (10% knockout serum [KSR] in iPSBrew).

NOTE: For optimal outcome, it is essential to use fresh cells.

2 Pellet cell by centrifuging them @ 400 x g for 00:05:00 . 5m

3 Count cells and harvest 500 000 cells per condition (and antibody).

EXAMPLE: If you are running a CRISPRi experiment (CRISPRi gRNA hiPSCs & non-targeting control hiPSCs) and you want to perform a Cas9 CUT&RUN on both conditions, you will harvest 500 000 CRISPRi and 500 000 control hiPSCs. If you are also including CUT&RUN controls (e.g. IgG control), you will need 500 000 per condition, per antibody- in this case: 500 000+500 000 CRISPRi hiPSCs & 500 000+500 000 control hiPSCs.

CUT&RUN (crosslinked)

2h 23m

4 Activate ConA-coat magnetic beads (Epicypher) by washing twice in Bead Binding Buffer [20 mM HEPES pH 7.5, 10 mM KCl, 1 mM CaCl, 1 mM $MnCl_2$]. Place on ice until use.

5 Centrifuge cells at 600 x g for 00:03:00 and resuspend the pellet in 1 ml of 0.1% formaldehyde solution to allow light crosslinking. Incubate for 00:01:00 at Room temperature . 4m

NOTE: Crosslinking is performed to capture more challenging targets such as Cas9, but it is not needed for the CUT&RUN control samples (e.g. IgG). To perform a regular CUT&RUN, refer to the the standard CUT and RUN protocol: dx.doi.org/10.17504/protocols.io.j8nlkwb8dl5r/v1

CAUTION: Formaldehyde is classified as a carcinogen and can cause respiratory and skin irritation upon exposure. Handle under a chemical hood and with the relevant protection equipment.

6 Add 125 mM of glycine and centrifuge again at 600 x g for 00:03:00 . 3m

7 Remove supernatant, and resuspend cells in 800 µL of XL-Wash Buffer (20 mM HEPES pH 7.5; 150 mM NaCl; 0.5 mM spermidine; 1 Roche Complete Protease Inhibitor EDTA-free tablet; 1% Triton X-100; 0.05% SDS - all diluted in dH_2O).

8 Centrifuge cells at 1000 x g for 00:03:00 and resuspend again in XL-Wash Buffer. Repeat two more centrifugations at 1200 x g (3 minutes each). 3m

9 Bind nuclei to beads (10 µL) for 00:10:00 at RT with gentle rotation, and then split bead-bound nuclei into three equal volumes (here, corresponding to IgG control and Cas9 antibody treatment). 10m

10 Remove wash buffer and resuspend nuclei in 100 µL cold XL-Antibody Buffer (XL-Wash Buffer; 0.05% digitonin; 2 mM EDTA; 1:50 anti-Cas9 antibody).

CAUTION: Digitonin is toxic. Gloves should be worn when preparing and working with digitonin.

11 Incubate Overnight at 4 °C on gentle shake. 3m

12 On the following day, remove supernatant by placing the sample(s) on a magnetic stand, and resuspend/wash the beads twice in XL-Digitonin Buffer (XL-Wash Buffer + 0.05% digitonin).

13 After the final wash, add pA-MNase (final concentration 700 ng/ml) in XL-Digitonin Buffer and incubate with the cells at 4 °C for 01:00:00. Wash nuclei twice, resuspend in 100 µL XL-Digitonin Buffer, and chill to 0 °C - 2 °C in a metal block sitting on wet ice. 1h

14 Stimulate genome cleavage by addition of 2 mM $CaCl_2$ at 0 °C for 00:30:00. Quench the reaction by adding 100 µL 2x Stop Buffer (0.35 M NaCl; 20 mM EDTA; 4 mM EGTA; 0.05%
digitonin; 50 ng/ml of RNAse A; 50 ng/ml of glycogen; 10 ng/ml spike in DNA; all diluted in distilled H_2O) and vortex. 30m

15 Incubate at 37 °C for 00:30:00 to allow the release of the fragmented chromatin. 30m

16 Place bead-bound nuclei on the magnet stand and collect the supernatant in a new 1.5 ml Eppendorf tube.

17 Add 0.09% SDS and 0.22 mg/ml of Proteinase K to reverse the crosslinking.

18 Isolate the DNA using NucleuSping PCR Cleanup columns (Macherey-Nagel) and prepare libraries for sequencing.

Sequencing

19 Prepare Illumina sequencing libraries using the Hyperprep kit (KAPA) with unique dual-indexed adapters (KAPA).