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CUT and RUN - Fresh cells

Forked from CUT and RUN

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anita.adami[1], Laura Castilla-Vallmanya[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.

Laura Castilla-Vallmanya
Lund University

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We use this protocol and it's working

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Abstract

This protocol describes how to perform CUT&RUN on fresh iPSC cells (optimisation might be required for other cell types).

Harvest and preparation of fresh cells

5m

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. Handle them carefully to avoid stress.

2 Pellet cell by centrifuging them at 400xg for 00:05:00 . 5m

3 Resuspend in media, count cells and harvest 500 000 cells per condition (antibody).

EXAMPLE: If you are running a CRISPRi experiment (CRISPRi gRNA hiPSCs & non-targeting control hiPSCs) and you want to perform H3K9me3 CUT&RUN on both conditions, you will harvest 500 000 CRISPRi and 500 000 control hiPSCs. Since you also need to include CUT&RUN controls (e.g. IgG control), you will need 500 000 more per condition- In total: 1 000 000 CRISPRi hiPSCs & 1 000 000control hiPSCs.

CUT&RUN

1h 53m

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 600xg for 00:03:00 . 3m

6 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 dH2O).

7 Repeat washing steps 5 & 6 2 more times.

8 Add 10 µL of pre-activated ConA-coated beads per condition (i.e. 30ul if using 3 different antibodies)

9 Bind cells to beads for 00:10:00 at RT on and end-over-end rotator, and then split bead-bound cells into three equal volumes (corresponding to IgG control, H3K4me3 and H3K9me3 treatments). 10m

10 Remove wash buffer and resuspend nuclei in 100 µL cold nuclear antibody buffer (20 mM HEPES pH 7.5, 0.15 M NaCl, 0.5 mM Spermidine, 1x Roche complete protease inhibitors, 0.02% w/v digitonin, 0.1% BSA, 2 mM EDTA) containing primary antibody at 1:50 dilution and incubate at 4 °C Overnight with gentle shaking. 10m

11 Wash nuclei thoroughly with nuclear digitonin wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, 1x Roche cOmplete protease inhibitors, 0.02% digitonin, 0.1% BSA) on the magnetic stand.

12 After the final wash, add pA-MNase in nuclear digitonin wash buffer and incubate with the nuclei at 4 °C for 01:00:00. Wash nuclei twice, resuspend in 100 µL digitonin buffer, and chill to 0 °C - 2 °C in a metal block sitting in wet ice. 1h

13 Stimulate genome cleavage by addition of 2 mM $CaCl_2$ at 0 ºC for 30 min. Quench the reaction by additing 100 µL 2x stop buffer (0.35 M NaCl, 20 mM EDTA, 4 mM EGTA, 0.02% digitonin, 50 ng/µL glycogen, 50 ng/µL RNase A, 10 fg/µL yeast spike-in DNA) and vortex.

14 Incubate 00:30:00 at 37 °C to release genomic fragments. Place bead-bound nuclei on the magnet stand and purify fragments from the supernatant using a NucleoSpin clean-up kit (Macherey-Bagel). 30m

Sequencing

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