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CUT and RUN

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We use this protocol and it's working

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Abstract

This protocols describe how to perform CUT&RUN on human brain tissue (frozen)

Troubleshooting

Sample extraction

1 Flash-freeze postmortem brain tissue

2 Sample 50 mg - 100 mg from human brain tissue and store at -80 °C until use

CUT&RUN

3 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.

4 **Isolate nuclei from frozen tissue** after incubating with Recombinant Alexa Fluor® 488 Anti-NeuN antibody [EPR12763] - Neuronal Marker (ab190195) at a concentration of 1:500 for 30 minutes on ice.
Run nuclei through the FACS at 4 °C with low flowrate using a 100 mm nozzle and isolate 300.000 nuclei Alexa Fluor – 488 positive nuclei.

5 Pellet the sorted nuclei at 1,300 x g for 00:15:00 and resuspend in 1 mL of ice-cold nuclear wash buffer (20 mM HEPES, 150 mM NaCl, 0.5 mM spermidine, 1x cOmplete protease inhibitors, 0.1% BSA) and 10 µL per antibody treatment of ConA-coated magnetic beads (Epicypher) added with gentle vortexing (Pipette tips for transferring nuclei were pre-coated with 1% BSA). 15m

6 Bind nuclei to beads for 00:10:00 at RT with gentle rotation, and then split bead-bound nuclei into three equal volumes (corresponding to IgG control, H3K4me3 and H3K9me3 treatments). 10m

7 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

8 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.

9 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

10 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.

11 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

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